The Effect of Atmospheric Sulfate Deposition on Mercury Biogeochemistry in an Experimental Peatland: Impacts, Recovery, and Natural Variability

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Dedication

This thesis is dedicated to my family for all of the emotional, spiritual, and sometimes physical support that they have given me during the long journey toward this goal.

My parents have put more faith in me than any one person could ever deserve. They provided the foundation that has allowed me to tackle challenges, reach for opportunities, and pursue dreams with confidence because I know that I always have a safe harbor no matter the storm.

My worldly-wise brother originally planted the seed in my head that my good fortune compels me to make a difference in the world.

My husband has given me the gift of his patience and has preserved the bright vision of our future when my head was buried in the peat. He reminds me to look up so I don't miss all the good stuff.

Abstract

Elevated mercury deposition resulting from human activities has caused wide-spread mercury contamination of aquatic systems around the world. Peatlands are generally considered to be sinks for mercury deposited to the landscape, but also act as biogeochemical reactors wherein inorganic mercury is transformed into bioaccumulative, organic methylmercury (MeHg). Recent, short-term investigations have demonstrated that sulfate deposition alone can increase MeHg production in, and flux from, peatlands through the stimulation of sulfate-reducing bacteria, a group of known mercury methylators. However, over longer periods of time the interaction between the biogeochemical cycles of mercury and sulfur is complicated by variability in climate, hydrology, and sulfur and mercury deposition rates.

These complexities were addressed by experimentally altering sulfate-loading to a 2.5-ha peatland in northern Minnesota over eight years. The peatland was initially divided into control and experimental treatments and sulfate was added to the latter three times each field season in simulated rainfall events. Porewaters were sampled before and after each sulfate addition and peat samples were collected five times from sites located within the raised central bog and along the peatland margins. The lagg margin is generally considered to be the primary site of mercury methylation in peatlands. However, sulfate addition caused more pronounced and persistent increases in MeHg in the central bog sites, relative to the margin sites, demonstrating that sulfate delivery to the central bog can greatly expand the areal extent of mercury methylation in peatlands. MeHg production also responded to sulfate release following severe summer drought.

The increase was much higher in experimental-treatment sites than in control sites suggesting that the experimental treatment was "primed" to quickly respond to new sulfate inputs.

In early 2006 sulfate addition was halted to the upgradient one-third of the original experimental treatment in order to monitor how MeHg production changed as sulfate deposition declined. Although drought appeared to slow the recovery process by increasing sulfate availability and mobilizing MeHg, three years after sulfate additions ceased MeHg in the recovery treatment was significantly lower than in the experimental treatment. This indicates that MeHg production in peatlands formerly affected by elevated sulfate deposition may return to background conditions and highlights the potential benefits that further controls on atmospheric sulfur emissions may have on MeHg production in peatlands and consequent mercury burdens in aquatic foodwebs. The long-term nature of this study allowed for an in-depth exploration of the effects that hydrologic flucutations on mercury cycling in peatlands and calls attention to the potential negative consequences that changing precipitation patterns and evapotranspirative demands may have on MeHg production in these systems.

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List of Abbreviations

Hg_T = Total mercury

MeHg = Methylmercury

%MeHg = Percent methylmercury or the fraction of the total mercury pool comprised by methylmercury

SO_4^{2-} = Sulfate

DOC = Dissolved Organic Carbon

S6 = Experimental wetland 6 in the Marcell Experimental Forest

WTE = Water table elevation

MEF = Marcell Experimental Forest

NADP = National Atmospheric Deposition Program

MDN = Mercury Deposition Network

Chapter 1

Introduction: The effect of atmospheric sulfate deposition on mercury biogeochemistry in an experimental peatland

1.1 Background

Mercury has long been a contaminant of concern because of myriad consequences to the health of humans and wildlife (Mergler et al., 2007; Munthe et al., 2007). Of particular interest to mercury contamination in terrestrial and aquatic foodwebs are the biogeochemical processes whereby bioaccumulative organic methylmercury (MeHg) is formed in and transported through the environment. Research over the past three decades has shown that different bacterial groups found in aquatic environments are capable of methylating inorganic mercury (Parks et al., 2013). Sulfate-reducing bacteria (SRB) are well-known mercury methylators in anoxic environments and their activity may be stimulated by an increased availability of sulfate (Benoit et al., 2002; Benoit et al., 1999; Gilmour et al., 1992). Laboratory, mesocosm, and field-scale studies have demonstrated that in certain systems, such as ombrotrophic peatlands, sulfate inputs can lead to increased net MeHg production and consequently higher mercury burdens in local biota than might be expected based on mercury inputs alone (Branfireun et al., 2001; Branfireun et al., 1999; Gilmour and Henry, 1991; Gilmour et al., 1998; Jeremiason et al., 2006; Swain and Helwig, 1989). To explore the role of variable sulfate inputs and

hydrology in MeHg production at an ecosystem scale sulfate-loading was experimentally manipulated to a small boreal peatland in the Marcell Experimental Forest (MEF) in northern Minnesota between 2001 and 2008.

1.2 Study design

In the summer of 2001 the S6 peatland was divided into an up-gradient control and a down-gradient experimental treatment. A PVC rainfall simulator was constructed across the experimental treatment and sodium sulfate solution was sprayed onto the peatland three or four times annually for seven full years with the goal of increasing atmospheric sulfate deposition by a factor of four times ambient, average 1990s rates. Peatland porewaters were sampled in the control and experimental treatments before and after each sulfate addition to monitor short-term changes in MeHg production resulting from the manipulation of sulfate loading. Because most mercury in a peatland is associated with the solid phase, peat samples were also collected five times throughout the course of the experiment to understand long-term changes in MeHg production. In 2006, after four-and-a-half years of experimental manipulation, sulfate additions were halted to the up-gradient, one-third of the original experimental treatment in order to monitor how/whether a peatland that had been affected by chronically elevated sulfate deposition would return to background conditions. At the same time depth-nested oxidation-reduction potential probes were installed in each treatment to record *in situ* redox conditions during seasonal water table fluctuations. In the spring of 2009 mercury burdens were quantified in mosquito larvae collected from each treatment as a means of

assessing how observed changes in MeHg production resulting from sulfate addition translated into effects on the local food web. Continuous data for hydrologic variables, including water table elevation, outflow, and upland runoff, were collected at long-term monitoring sites in the watershed and provided by the US Forest Service. Atmospheric mercury and sulfate deposition data were obtained from the Mercury Deposition Network and National Atmospheric Deposition Program, respectively, for the long-term, atmospheric deposition monitoring sites located in the MEF (NADP, 2014).

1.3 Summary of findings

1.3.1 Effect of chronic sulfate addition on MeHg production

Sulfate addition caused short and long-term changes in MeHg production in the experimental treatment, particularly in the ombrotrophic center. Generally MeHg concentrations and %MeHg (fraction of total mercury (Hg_T) present as MeHg) in the porewaters of the experimental treatment reached peak values within a week of sulfate addition and then declined as the added sulfate disappeared. Average MeHg levels in the experimental treatment were always greater than corresponding levels measured in the control treatment suggesting a chronic stimulation of net methylation. Experimental treatment MeHg and %MeHg increased cumulatively over time in the solid-phase peat, which acted as a sink for newly produced MeHg. Hg_T levels in mosquito larvae collected from the experimental treatment were more than two times greater than in the control suggesting that the newly produced MeHg was bioavailable.

Sulfate addition increased the pool of MeHg stored in the S6 peatland primarily through its effect on net mercury methylation in the central bog. Hydraulic gradients in the S6 peatland effectively isolate the raised central bog from mineral and nutrient inputs associated with upland runoff. This causes sulfur-limitation in the central bog, which results in a degree of natural spatial variability in MeHg production across the system. At ambient sulfate-loading MeHg in porewaters and solid-phase samples were highest in the narrow peatland margin, or lagg, likely because sulfate inputs from uplands stimulated mercury methylation. By the end of the study chronic elevation of atmospheric sulfate deposition had caused a 5-6 fold increase in MeHg concentrations and %MeHg levels in the central bog of the experimental treatment relative to control values. In contrast no difference in solid-phase MeHg levels was measured between control and experimental treatment lagg sites. While this indicates that the extra sulfate did not increase net MeHg production in the lagg on an annual basis, observed increases in MeHg levels in lagg porewaters after sulfate additions and greater Hg_T burdens in mosquito larvae collected from experimental treatment lagg sites relative to control sites suggest that net mercury methylation was at least stimulated seasonally in the lagg. Because sulfate inputs to the lagg are naturally higher than to the central bog it may be that other processes, such as MeHg demethylation, were more important to the size of the long-term MeHg pool in the lagg than the intermittent doses of sulfate from this experiment.

1.3.2 The dual role of hydrology in MeHg production

While elevated atmospheric sulfate deposition caused a significant build-up of the MeHg pool in the central bog, the contribution of the central bog to MeHg flux from this

peatland is ultimately an issue of hydrology. Over the course of a season the water table can fluctuate as much as 40 cm because the S6 peatland is perched above the regional groundwater table. Water running off of the central bog mound and surrounding uplands flows to the lagg margin and then toward the outflow. At high water table elevation (WTE) hydraulic gradients between the central bog and lagg are maximized and water moves quickly through shallow, relatively undecomposed peat layers (Gafni and Brooks, 1990). Conversely at low WTE advective flow is inhibited in deeper, decomposed peat layers, hydraulic gradients are not as steep, and the central bog becomes somewhat disconnected from peatland outflow. Climate patterns that cause hydrologic fluctuations may therefore directly affect MeHg flux by increasing or decreasing hydrologic connectivity between the central bog and peatland outflow. Furthermore fluctuating water tables affect the gradient of oxidation-reduction potentials within the peat profile with consequent changes to chemical speciation and adsorption dynamics of sulfur and mercury in porewaters and solid peat.

Several droughts of variable intensity during the course of this project provided the opportunity to study the effects of large hydrologic fluctuations on mercury cycling in peatlands experiencing differing rates of atmospheric sulfate deposition. Generally WTE followed a similar pattern from year to year. WTE was highest during the spring season after snowmelt, declined over the summer period as evapotranspirative demands increased, and rebounded in the fall after vegetation senescence. Not surprisingly peatland outflow (a proximal indicator of hydrologic connectivity) was directly related to WTE, and oxidation-reduction potentials were inversely related to WTE. Summer

droughts in 2005, 2006, and 2007 allowed the upper 25-40 cm of peat to oxidize in the S6 peatland and resulted in mobilization of sulfate, MeHg, and Hg_T from the solid phase as the water table rose during fall rebound or following large precipitation events. Sulfate releases were high across the peatland, but were greatest in peat that had been recently affected by elevated rates of sulfate deposition indicating recycling of previously sequestered sulfate. Similarly MeHg was elevated because of oxidative release from the solid phase as well as increased mercury methylation resulting from stimulation of SRB activity by newly available dissolved sulfate. Hg_T concentrations also increased in peatland porewaters following water table rises, but release did not appear to be affected by past sulfate inputs. Thus large-scale fluctuations in WTE caused by severe drought and intense precipitation events may reduce the role of peatlands as sinks for sulfate and inorganic mercury in the landscape while at the same time increasing their strength as sources of MeHg to downstream aquatic systems through the additive effects of oxidative MeHg release and sulfate-stimulated MeHg production.

1.3.3 The effect of declining sulfate deposition on MeHg production

The finding that chronically elevated sulfate deposition altered MeHg production dynamics in the S6 peatland through the creation of a pool of reduced sulfur compounds that were recycled during water table fluctuations raises the question of whether systems impacted by many years of elevated sulfate deposition can return to a pre-impact state. Historically atmospheric sulfate deposition rates were more than an order of magnitude greater than background across large portions of the northeastern United States, eastern Canada, and eastern and northern Europe (NADP, 2014; Schopp et al., 2003; Stern,

2006). The implementation of sulfur emissions controls through policies, such as the 1990 Clean Air Act Amendments in the United States, were intended to address the more widely recognized problem of ecosystem acidification. Despite significant reductions of atmospheric sulfate deposition across many regions resulting from such policies ecosystems have been slow to respond (Keller et al., 2003; Prechtel et al., 2001; Stoddard et al., 1999). Because the relationship between MeHg production and sulfate was discovered well after the acidifying effects of sulfate raised concerns, no data exist that allow for the direct assessment of changes in MeHg production resulting from large scale declines in sulfate deposition following the implementation of regulations. Furthermore altered precipitation patterns caused by a changing climate may actually increase sulfur recycling in peatlands leading to higher MeHg production regardless of declines in sulfate deposition.

Observations from the recovery treatment created mid-way through this study indicate that MeHg production in peatlands previously impacted by high levels of sulfate deposition start to return to background levels relatively quickly. In 2006, the year experimental sulfate deposition was discontinued, annual seasonally-weighted MeHg levels in recovery treatment porewaters and peat were not significantly different from levels observed in the experimental treatment. Although annual MeHg concentrations and %MeHg levels in porewaters and peat of the recovery treatment were still elevated relative to the control by 2008 they were significantly lower than levels observed in the experimental treatment. Furthermore mosquito larvae collected from each treatment at the end of the experiment exhibited Hg_T concentrations strongly correlated with MeHg

levels in the peat and porewaters where they were collected, i.e. Hg_T levels were significantly lower in mosquito larvae collected in the recovery treatment than in the experimental treatment. While drought appeared to slow the recovery process through sulfate release, it appeared that the added sulfate was eventually incorporated into more recalcitrant sulfur pools because sulfate release following drought and rewetting cycles declined over time in the recovery treatment. The relatively rapid declines in MeHg throughout the porewater, solid phase, and biotic compartments in the recovery treatment in response to lower sulfate deposition suggests that further controls on sulfur emissions may represent an additional means of mitigating Hg contamination in fish and wildlife across low-sulfur landscapes.

Chapter 2

Spatially variable response of mercury methylation to sulfate addition in a boreal peatland

2.1 Summary

An eight-year, ecosystem-scale sulfate addition experiment in northern Minnesota provided an opportunity to study how elevated levels of atmospheric sulfate deposition affect the spatial distribution of methylmercury (MeHg) production in boreal peatlands. At ambient sulfate loading the narrow lagg-margin between the ombrotrophic central bog and surrounding uplands was found to be the primary site of MeHg production. However, when sulfate loading was experimentally raised to 4X ambient the zone of high net Hg methylation expanded into the relatively large center of the peatland. Chronically elevated sulfate deposition caused cumulative, annual increases in solid-phase %MeHg (fraction of total-Hg (Hg_T) present as MeHg) in the central bog, thereby increasing the pool of MeHg potentially available for export to downstream aquatic systems. No long-term effect of sulfate addition was observed on net Hg methylation in the lagg margin, perhaps as a result of high demethylation rates in the alder-lagg peat. However, %MeHg levels rose in lagg porewaters after experimental sulfate additions and higher Hg_T burdens in mosquito larvae in experimental treatment lagg sites, relative to control sites,

suggest that sulfate addition stimulated Hg methylation in lagg sites long enough to allow newly produced MeHg to enter the local foodweb. While mechanisms that increase sulfate delivery to the raised central bog (e.g. atmospheric sulfate deposition, water table fluctuations following peat oxidizing droughts) have the potential to enhance MeHg production and storage in peatlands, the contribution of the central bog to MeHg flux from these systems will ultimately be controlled climate patterns that affect hydrologic connectivity between the central bog and peatland outflow.

2.2 Introduction

Boreal peatlands provide environmental conditions highly conducive to the bacterial production of neurotoxic methylmercury (MeHg) and are considered to be among the most important sources of MeHg to food webs in adjacent terrestrial and downstream aquatic systems (St. Louis et al., 1994; Wiener et al., 2006). While various bacteria are known or suspected mercury (Hg) methylators (Parks et al., 2013) numerous studies have demonstrated a key role for sulfate reducing bacteria (SRB) – showing that conditions favorable to SRB communities, such as available sulfate and anoxia, are also favorable to MeHg production (Branfireun et al., 2001; Branfireun et al., 1999; Coleman Wasik et al., 2012; Gilmour et al., 1992; Gilmour et al., 1998; Jeremiason et al., 2006). This understanding has lead to various efforts to better define when and where Hg methylation is most favored in peatland ecosystems (Alpers et al., 2014; Mitchell et al., 2008b, 2009; Tjerngren et al., 2012b; Windham-Myers et al., 2014).

Recent research points to the influence of watershed hydrology in creating zones of high methylation potential ("hot spots") along the wetland-upland margin of sulfur-limited peatlands (Mitchell et al., 2008b, 2009). These Hg-methylation hot spots result from the delivery of nutrients and solutes from the mineral-soil upland to the anoxic, organic-rich wetland. Moreover, this wetland margin (lagg) often represents the dominant hydrologic flow path in small peatland systems, such that zones of elevated Hg methylation in the lagg may also contribute disproportionately to peatland MeHg export. However, exclusive focus on localized hot spots at the lagg margin may underestimate the importance of MeHg production in the central raised-bog portion of the peatland. A growing body of research has demonstrated that sulfate addition to the ombrotrophic center of sulfur-limited peatlands can result in order-of-magnitude increases in MeHg in porewaters and 2-6 x increases in solid-phase and biotic MeHg (Åkerblom et al., 2013; Branfireun et al., 2001; Branfireun et al., 1999; Coleman Wasik et al., 2012; Jeremiason et al., 2006; Mitchell et al., 2008a). Therefore it is important to consider processes that increase sulfate delivery to the much larger central area of the peatland, such as atmospheric deposition and sulfate-release following drought, both of which may greatly expand the areal extent of high net methylation.

An eight-year, ecosystem-scale, sulfate-addition experiment in a northern Minnesota peatland created an opportunity to examine how spatial variability in MeHg production within peatlands changes in response to both elevated atmospheric sulfate deposition and year-to-year hydrologic change. For this experiment the peatland was divided into treatments that received differing loads of atmospheric sulfate via simulated

rainfall. Our previous reports from this study documented both short-term and chronic effects of elevated sulfate deposition on MeHg concentrations within and export from the peatland as well as recovery once rates of sulfate loading were reduced (Coleman Wasik et al., 2012; Jeremiason et al., 2006). Here we extend this account to examine how such effects are distributed spatially within the wetland based on an intensive collection of porewaters, peat cores, and biotic indicators (aquatic invertebrates) from lagg margin and central bog sites Our main objectives are to: 1) describe spatial variability in MeHg production across a peatland at ambient rates of atmospheric sulfate deposition, 2) determine how increased sulfate-loading to the entire system alters the natural patterns in MeHg production, and 3) translate changes in the spatial variability of MeHg production to alterations in potential MeHg flux from the peatland.

2.3 Methods

2.3.1 Study site

This study was carried out in the S6 wetland (47.5206° N, 93.4713° W) of the Marcell Experimental Forest (MEF) – U.S. Forest Service, Northern Research Station – in northern Minnesota, (Figure 2.1). This 2.0-ha forested peatland occupies an elongate, morainal depression and is surrounded by a 6.9-ha white spruce (*Picea glauca*) and red pine (*Pinus resinosa*) upland (Sebestyen et al., 2011). The peatland overstory is dominated by mature black spruce (*Larix laricina*) and tamarack (*Picea mariana*) in the central bog and speckled alder (*Alnus rugosa*) along the lagg margins (USDA-USFS, 2014). The ombrotropic nature of the raised central bog results in a strong trophic

gradient from the mineral-poor center toward the mineral-rich margins (Mitchell et al., 2008b). Water flows predominantly from the raised center and surrounding uplands to the lagg margin and discharges from a small outlet stream at the southeastern end of the peatland. Upland surface flow and interstitial flow collectors are used to estimate hydrologic inputs from the uplands, and outflow is monitored continuously at a 120°-notch weir.

2.3.2 Sulfate deposition experiment

Sulfate was experimentally added to the S6 peatland from the fall of 2001 through the fall of 2008. The peatland was initially divided into an up-gradient control and a down-gradient experimental treatment, and a rainfall simulator (irrigation sprinkler system) was constructed within the experimental treatment to deliver sulfate to the site (Figure 2.1). A 10-cm diameter PVC pipeline was laid along the northeastern edge of the peatland and thirteen 5-cm PVC lateral lines extended from the main pipeline across the experimental treatment. Sprinkler heads installed on 1-m risers at regular intervals along each lateral line evenly distributed the sulfate solution across the experimental half. Atmospheric sulfate deposition, as recorded at a nearby National Atmospheric Deposition Program (NADP) site (MN-16), had declined by ~50% at MEF from 11 kg ha^{-1} yr^{-1} in the early 1980s to ~5.5 kg ha^{-1} yr^{-1} in the mid-2000s (NADP, 2014). Sulfate additions increased annual sulfate loading to 32 kg ha^{-1} yr^{-1}, approximately 4x the average ambient deposition rate at MEF in the 1990s, allowing us to simulate sulfate loads experienced by peatland ecosystems across large regions of the northeastern United States and adjacent Canada throughout the late 20^{th} century. A

recovery treatment was created in the spring of 2006 by halting sulfate additions to the up-gradient one-third of the original experimental treatment (Figure 2.1).

A sulfate-enriched solution was applied to the experimental treatment in three simulated rainfall events each field season. Low conductivity water (~20 μS cm^{-1}) was pumped from a nearby pond through the main pipeline, and a concentrated sodium sulfate solution was injected into the line just above the experimental treatment. A rinse period followed each sulfate addition to clear sulfate out of the lines and wash sulfate off of vegetation. Each sulfate addition and rinse simulated approximately 6-8 mm of rainfall and had minimal effects on water table elevation.

2.3.3 Field Sampling

2.3.3.1 Porewaters

Two sampling transects were initially established in the control and experimental treatments, and four 1-m^2 sample plots were distributed evenly between the central bog and lagg margin along each transect. In 2006 two transects were created in the new recovery treatment and the original experimental treatment transects were repositioned down-gradient to ensure that sampling occurred within the treated area. Peat porewater samples were collected on days -1, +1, +3, and +7 relative to each sulfate addition. Extra sampling was conducted on days -7 and +14 for each spring and fall sulfate addition and during periods of hydrologic interest in 2007 and 2008 (e.g. snowmelt, storm events).

Porewater samples were collected by first inserting a 1.9-cm ID Teflon probe (custom-made tip perforated with 5-mm holes) into the peat to a depth approximately 5 cm below the water table. Porewater was then extracted by peristaltic pump via 0.63-cm

ID Teflon tubing through acid-washed 47-mm diameter Teflon filter-holders (Savillex Co.) that were pre-loaded with ashed, 0.7-µm, glass-fiber filters. Porewater samples for dissolved Hg_T and MeHg were filtered directly into new, 125-mL, PETG bottles and handled using accepted clean-sampling techniques (Bloom and Fitzgerald, 1988). Bottles were triple-rinsed with porewater prior to filling and samples were preserved with high-purity HCl to 0.5% (v/v). Samples for dissolved Hg_T, MeHg, and major anions were collected on each sampling day throughout the project. Porewater samples were also collected for dissolved organic carbon (DOC) and major cations on the day before each sulfate addition in 2005 and 2006. DOC samples were collected on each sampling day in 2007 and 2008.

2.3.3.2 Peat Samples

Surficial peat cores were collected from bog and lagg locations within each treatment one time during 2003, 2005-2007, and 2009. Cores were collected using a McCauley, side-filling peat corer in 2005 and by cutting and hand-collection in 2003, 2006, 2007, and 2009. All peat samples were kept in frozen storage and freeze-dried prior to analysis. Samples from the 0-2, 2-4, and 4-8-cm intervals were considered in this study.

2.3.3.3 Invertebrate Samples

Mosquito (*Culex* spp.) larvae samples were collected in the late spring of 2009, near the end of this study. Larval samples were collected in triplicate batches from each location (bog and lagg) within each treatment by netting with vinyl-coated aquarium nets. Mosquito larvae were then hand-picked at the MEF laboratory, placed in vials of

deionized water overnight to purge gut contents, and frozen. Samples were freeze-dried prior to analysis of Hg_T content. Where sufficient mass remained, samples were also analyzed for MeHg content.

2.3.4 Analytical Methods

2.3.4.1 Dissolved mercury

Aqueous Hg_T was analyzed at the Branfireun laboratory in accordance with EPA method 1631, Revision E (US-EPA, 2002). Briefly, a 50-mL aliquot of each sample oxidized overnight by bromine monochloride (BrCl) additions to convert all mercury species to Hg(+II). Hydroxylamine hydrochloride (NH_2OH-HCl) was added to neutralize any remaining BrCl and Hg (+II) was reduced to Hg (0) by stannous chloride ($SnCl_2$) addition. Gaseous Hg^0 was purged from solution and captured on gold-coated glass beads in a "sample" trap, thermally desorbed, and trapped again on an "analytical" trap. Finally the Hg was thermally desorbed a second time into a stream or argon gas and analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS) on a Tekran 2600 Automated Total Mercury Analyzer. Instrument calibration was checked daily with lab-made standards and each analytical run included 20% deionized water blanks, 10% sample duplicates, and 5% sample matrix spikes.

Aqueous MeHg was by the Branfireun lab (2005), the Jeremiason lab (2006), and the Balogh lab (2007 and 2008) analyzed according to the methods described in Bloom (1989) and Liang et al. (1994). In each lab forty-mL aliquots of sample were amended with 0.2 mL of potassium chloride and 1 mL of sulfuric acid. Samples were heated to 135°C under ultrapure nitrogen gas until 80% of the sample had distilled into the

receiving vial. Distillates were refrigerated and analyzed within 48 hours by cold vapor atomic fluorescence spectroscopy (CVAFS) with chromatographic separation. Distillates were buffered to a pH of 4.9 using an acetic acid/sodium acetate solution and Hg species were then ethylated with sodium tetraethylborate (NaTEB) before being purged from solution and trapped on Tenax traps. Mercury species were thermally desorbed from the traps and carried in a stream of argon through a short chromatographic column. The separated Hg species passed through a pyrolytic trap and thermally transformed into Hg^0 prior to entering the Tekran 2500 (Branfireun and Jeremiason labs) or Brooks Rand Model III (Balogh lab) CVAFS spectrometer.

Instrument calibration was checked daily with lab-made standards and each analytical run included 5% deionized-water blanks, 10% sample duplicates, and 5% sample matrix spikes. For Hg_T and MeHg analyses, poor calibration linearity or quality control samples more than 15% out of range precluded sample analysis until the analytical issue was resolved. QAQC results are included in Appendix A, Tables A1 (Hg_T) and A2 (MeHg).

2.3.4.2 Solid-phase mercury

For Hg_T analysis, peat samples were microwave digested in concentrated HNO_3 and diluted prior to analysis by dual gold-trap amalgamation CVAFS, as described above for porewaters. For MeHg analysis, peat samples were distilled as outlined for porewaters, but with the inclusion of a known mass spike of enriched $Me^{199}Hg$ in each vessel. Samples were analyzed by isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry (ID-GC-ICPMS) with detection on an Agilent 7700

ICPMS according to the methods of Hintelmann et al.(1995) In addition to blanks and duplicates, certified reference materials (MESS-3 for Hg_T; ERM-CC580 for MeHg) were analyzed in 10% of samples. QAQC results for Hg_T and MeHg in solid phase samples are included in Appendix A, Table A3.

2.3.4.3 Biological mercury

For Hg_T analysis, mosquito larvae samples were microwave digested in concentrated HNO_3 and diluted prior to analysis by dual gold-trap amalgamation CVAFS, as described for porewaters. MeHg in mosquito larvae samples was heat extracted in a solution of 25% KOH in methanol, with a known mass spike of enriched $Me^{199}Hg$ in each vessel. Samples were analyzed by ID-GC-ICPMS. In addition to blanks and duplicates, the certified reference material DORM-3 was analyzed in 10% of samples.

2.3.4.4 Major Ions

All water samples for major anions were analyzed according to standard methods by the USFS Northern Research Station laboratory using single column, suppressed ion chromatography on a DX-500 ion chromatograph. A guard column was inserted into the manifold prior to the exchange column to remove column-fouling organic compounds. Anions were detected by a conductivity cell and PeakNet 5.0 software was used to resolve and integrate the resulting peaks. Replicate standard measures and lab duplicates were within 10%. Method detection limits were 0.1 mg L^{-1} each year.

Major cation samples were analyzed at the University of Minnesota's geochemistry lab. Samples collected in 2005, 2006, and 2007-2008 were analyzed

according to standard methods using inductively-coupled plasma mass spectrometry, ion chromatography, and inductively-coupled plasma optical emission spectrometry, respectively. Duplicates comprised 20% of each analysis set and standards and blanks comprised another 25% of each analysis set.

2.3.4.5 Dissolved Organic Carbon

Dissolved organic carbon was analyzed by either UV-persulfate digestion on a Tekmar-Dohrmann Phoenix 8000 Carbon Analyzer (St. Croix Watershed Research Station) or by combustion with catalytic oxidation on a Shimadzu Carbon Analyzer (Jeremison lab). In both methods inorganic carbonates were removed from samples by an acid pre-treatment. The remaining organic carbon was then either exposed to sodium persulfate in the presence of a UV light (UV-persulfate method) or catalytically oxidized at 680°C in a high temperature furnace (Combustion method). In each case the resulting carbon dioxide evolved from samples was measured by non-dispersive infrared detection. Check standards, calibration verifications, and blanks comprised 15% of each analysis. Replicate standard measures and lab duplicates were within 10% and method detection limits were 0.1 mg L^{-1} each year.

2.3.5 Numerical analyses

Mean porewater sulfate and Hg concentrations in each location and treatment were calculated for each sampling date. Mean values for each peat core were calculated by multiplying the concentration for each interval by a weighting factor related to interval thickness (2 or 4 cm) and summing (e.g. the 0-2 cm interval received less weight than the 4-8 cm interval). Treatment and location means were then calculated from the weighted

averages. Mosquito larvae results from each sample batch were averaged for each treatment and location.

All statistical analyses were performed using the statistical software package R (R-Development-Core-Team, 2011). The distributions for both porewater and solid-phase data were right-skewed, so each data set was natural-log-transformed prior to statistical analyses to obtain a normal distribution. Kruskal-Wallis Rank Sum analyses were used to assess the effect location and treatment on average daily Hg and sulfate concentrations in porewaters and Hg in mosquito larvae. The Wilcoxon Signed-Rank analysis was then used to make detailed comparisons of mean porewater, solid-phase, and mosquito-larvae sulfate and Hg concentrations among treatments and locations (bog vs. lagg). Simple bi-plots were created to visualize the average difference between daily mean sulfate, Hg, or DOC porewater concentrations in bog and lagg locations. Simple linear regressions for each treatment indicated the average daily difference between bog and lagg locations for each parameter. P-values less than 0.05 were considered to be significant.

2.4 Results

2.4.1 Porewater Trends

2.4.1.1 Sulfate

Porewater sulfate concentrations in the S6 peatland varied by season, treatment, and location (bog vs. lagg), as illustrated by the results from the 2008 field season (Figure 2.2). Seasonal patterns were marked by moderately high sulfate concentrations in

snowmelt (2 mg L^{-1}), low concentrations (< 0.25 mg L^{-1}) during the spring and summer seasons, and elevated concentrations (0.5-12 mg L^{-1}) during the fall (Figure 2.2a). Very high sulfate concentrations for this system (> 5 mg L^{-1}) were also observed in porewaters during large water-table rises as sulfate was released from peat that had oxidized during drought conditions (Appendix A, Figure A1).

Average daily sulfate concentrations in porewaters of the control and recovery treatments were not significantly different on most sampling dates ($p > 0.05$ Wilcoxon Rank Sum). Sulfate applications to the experimental treatment caused transient increases, but otherwise sulfate concentrations in experimental treatment porewaters were often comparable to those observed in the control and recovery treatments. The effect of treatment was more noticeable on average annual sulfate concentrations, with the control treatment being the lowest (0.2 – 1.7 mg L^{-1}), the experimental highest (1.1 – 4.7 mg L^{-1}), and the recovery intermediate (0.6 – 2.4 mg L^{-1}). These annual differences were driven primarily by a relatively few, but very high sulfate concentrations observed in the recovery and experimental treatments as a result of sulfate release from the peat following severe droughts in 2006 and 2007 (Appendix A, Figure A1).

Location within the peatland (bog vs. lagg) was also an important determinant of sulfate concentration within and among treatments, and by season (Figure 2.2b, c). Porewater sulfate concentrations were generally higher at lagg sites than at bog sites for any given treatment or season. However, the differences in sulfate concentrations among treatments were most pronounced at bog sites.

2.4.1.2 Total mercury

Porewater Hg_T concentrations varied by season, location, and treatment within the peatland (Appendix A, Figure A2), but were primarily influenced by hydrologic conditions. Generally Hg_T concentrations were relatively low during the spring (4-6 ng L^{-1}) and rose through the summer and fall (to > 10 ng L^{-1}). Location was found to be a significant factor ($p < 0.05$ Kruskal-Wallis) with higher Hg_T concentrations observed at lagg sites than at bog sites. Treatment also had a significant effect on Hg_T concentrations ($p < 0.005$ Kruskal-Wallis) with higher concentrations generally found in the control treatment than in the experimental or recovery treatments. The greatest Hg_T concentrations (15-20 ng L^{-1}) were observed in porewaters following the droughts of summer 2005, summer/fall 2006, and summer 2007.

2.4.1.3 Methylmercury

Porewater MeHg concentrations were significantly influenced by both treatment and location within the S6 peatland, as illustrated by the results from the 2008 field season (Figure 2.3). MeHg concentrations during the snowmelt period were uniformly low (< 0.5 ng L^{-1}) across the peatland. After the peat had thawed in early May, MeHg concentrations rose in each treatment, peaked in mid-May, and then slowly declined. MeHg concentrations were similar between the control and recovery treatments throughout the remainder of the year. In contrast, MeHg concentrations in experimental treatment porewaters were 1.2-3x higher than those in the control and recovery treatments prior to sulfate additions during the spring, summer, and fall. Following sulfate additions MeHg concentrations in the experimental treatment rose by 2-4x from

pre-addition levels resulting in MeHg concentrations in experimental treatment porewaters that were 2-10x higher than the levels measured in the control and recovery treatments.

Location within each treatment was an important determinant of porewater MeHg concentrations, but the effect was not uniform among treatments (Figure 2.3b, c). In the control treatment MeHg concentrations were significantly higher at lagg sites than at bog sites ($p < 0.001$, Wilcoxon Rank Sum), whereas the reverse was true in the experimental treatment ($p < 0.05$, Wilcoxon Rank Sum). No significant difference in MeHg concentrations between bog and lagg sites was found for the recovery treatment ($p = 0.3$, Wilcoxon Rank Sum). As noted for sulfate concentrations above, the difference in MeHg concentrations among the treatments was greater at bog sites than at lagg sites.

Percent MeHg (%MeHg) – the fraction of the total pool of Hg comprised of MeHg – in porewaters was used as a proxy for short-term, net MeHg production across the peatland. For example, despite comparable MeHg concentrations in control and recovery treatment porewaters during 2008, %MeHg was often greater in the recovery treatment than in the control (Figure 2.3a) indicating higher levels of MeHg relative to Hg_T, and thus greater MeHg production, in the recovery treatment. Furthermore, the rise in MeHg concentrations during the early spring described above resulted in very large increases in %MeHg and represented a period of active MeHg production across the peatland.

Generally %MeHg levels were lowest in the control treatment, highest in the experimental treatment, and intermediate in the recovery treatment. The lowest %MeHg

levels were observed during the snowmelt period (all treatments) and the highest levels during late spring and early summer in the control and recovery treatments. %MeHg levels in the experimental treatment rose by a factor of 2-4x following the spring snowmelt and after each sulfate addition. As described for MeHg concentrations, %MeHg levels varied significantly by location. Although control lagg sites were again significantly higher than control bog sites ($p<0.001$; Wilcoxon Rank Sum), %MeHg levels in recovery treatment bog sites were significantly higher than in recovery treatment lagg sites ($p < 0.03$; Wilcoxon Rank Sum), and no significant difference was found in %MeHg levels among experimental treatment bog and lagg sites. Similar to MeHg concentrations, the differences in %MeHg among treatments was most pronounced at bog sites.

2.4.2 Spatial differences between bog and lagg

2.4.2.1 Porewaters

The distribution of individual samples with high %MeHg – those exceeding the 90th percentile for each treatment – varied spatially (Table 2.1; Appendix A, Table A4). In the control treatment 80% of these high values (%MeHg > 11%) were found in the lagg margin, even though two-thirds of all control samples collected between 2005 and 2008 were from the central bog. This situation was reversed in the experimental treatment where 92% of samples exceeding the 90th percentile (%MeHg > 41%) were collected from the central bog. Samples exceeding the 90th percentile in the recovery treatment (%MeHg > 20%) were more evenly distributed between lagg and bog and changed over time as MeHg concentrations declined. In 2006 89% of high-%MeHg samples were from

the bog, but that portion dropped to 69% in 2007 and 65% in 2008 – similar to the actual proportion of samples collected from the central bog (68% and 64%, respectively).

The differences in porewater chemistry between lagg and bog sites is further revealed by simple bi-plots of the average daily values of porewater sulfate, MeHg, Hg_T, and DOC from bog sites against corresponding daily values from lagg sites within each treatment (Figure 2.4). The linear least-squares fit through each set of points represents the average daily difference between bog and lagg sites among sampling days in each treatment (Appendix A, Table A5). In the control treatment the average sulfate concentration in the lagg was ~5x greater than that in the bog, while in the recovery treatment sulfate was ~3.5x greater in the lagg than the bog. In contrast, sulfate concentrations at experimental treatment bog sites were nearly equivalent to concentrations measured at lagg sites. Furthermore, sulfate concentrations at bog sites tended to be higher than at lagg sites (~1 mg L^{-1}) when daily sulfate concentrations were low (lagg sites < 0.5 mg L^{-1}).

Average MeHg concentrations and %MeHg in the lagg of the control treatment were both ~3-4x the corresponding levels measured in the bog. In the recovery treatment MeHg concentrations at lagg sites were ~3 times higher than at bog sites, while %MeHg levels at bog sites were generally equivalent to those at lagg sites. In contrast, MeHg concentrations in experimental treatment bog sites were ~1.5 times those measured at lagg sites and %MeHg levels were slightly greater at bog sites than at lagg sites. Hg_T concentrations were somewhat higher in the lagg than the bog, although the differences were not significant and did not vary by treatment. DOC concentrations at

bog sites were slightly higher than at lagg sites, but likewise did not vary systematically by treatment.

2.4.2.2 Solid phase MeHg

MeHg concentrations and %MeHg in peat samples varied spatially and temporally over the course of this study (Figure 2.5). Again the effect of sulfate additions on MeHg in the solid phase were most evident in the central bog. In 2003, experimental treatment MeHg concentrations and %MeHg levels were 2x higher than control values. By 2009, MeHg in the solid phase was 5-6x greater than control values. In 2006, recovery treatment MeHg concentrations and %MeHg in the central bog were 3-4x higher than control treatment values, but had declined to near-control values by 2009. MeHg concentrations and %MeHg levels in experimental and recovery treatment lagg sites showed little difference from control values over the course of study and no strong trends with time – in marked contrast to the large differences noted in the central bog.

2.4.2.3 Mosquito Hg_T

Total Hg burdens in mosquito larvae collected in the spring of 2009 from bog and lagg sites in each treatment reflected MeHg patterns similar to those noted for porewaters and peat samples (Figure 2.6). Overall larval Hg_T concentrations were significantly influenced by both treatment ($p<0.005$, Kruskal-Wallis) and location ($p<0.02$, Kruskal-Wallis). However, pairwise comparisons of larval Hg_T in bog and lagg sites within and among treatments were not significant ($p=0.1$, Wilcoxon Rank Sum), likely because of small sample size. At lagg sites, average Hg_T concentrations in the experimental treatment were nearly 50% greater than those in control sites, while the recovery

treatment was no different from the control. At bog sites, Hg_T levels in the experimental treatment were more than 2x greater than those in control bog sites while recovery treatment mosquito Hg_T were 36% higher than those in control sites three years after sulfate additions had ceased.

2.5 Discussion

2.5.1 Spatial variability in mercury methylation

2.5.1.1 Ambient sulfate deposition

Mercury and sulfate concentrations measured in this study were comparable to levels reported for other boreal peatlands in the region (Heyes et al., 2000; Kolka et al., 2001; Mitchell et al., 2008b; St. Louis et al., 1994). Mercury methylation in the control treatment was greater in the lagg margin than in the central bog as evidenced by higher solid phase and porewater MeHg concentrations and %MeHg levels at lagg sites than at bog sites (Figures 2.4, 2.5). Furthermore, distributional analysis of %MeHg levels across the control treatment indicated that a disproportionate number of high %MeHg values were measured at lagg sites compared to bog sites (Table 2.1) suggesting greater net MeHg production in the lagg under conditions of ambient sulfate loading. This finding agrees with previous research at S6 which found much higher %MeHg in peatland porewaters within 5 m of the upland-peatland interface (i.e. the lagg margin) than in porewaters collected > 5 m from the interface (Mitchell et al., 2008b).

Elevated MeHg production in the control lagg likely resulted from greater sulfate availability in the lagg as compared to the sulfate-limited central bog. Previous research

has demonstrated the stimulatory effect of sulfate addition on SRB activity and MeHg production in this peatland (Coleman Wasik et al., 2012; Jeremiason et al., 2006), and average daily sulfate concentrations in the present study were as much as 5x greater in lagg porewaters than in bog porewaters of the control treatment (Figure 2.4, Table 2.1). Upland runoff likely provides an important source of sulfate to the S6 lagg, as was found for a nearby peatland at MEF (Urban et al., 1989). Solid-phase total sulfur concentrations in the top 16 cm of peat were also greater at lagg sites than at bog sites in each treatment (Appendix A, Figure A3), indicating either an additional source of sulfur to the lagg margin or greater decomposition of organic matter, and concomitant sulfur mineralization (and therefore recycling), in the lagg relative to the bog (Novak et al., 1994).

2.5.1.2 Elevated sulfate deposition

Sulfate addition to the experimental treatment expanded the zone of intense net methylation from the lagg into the central bog. Mean porewater sulfate concentrations in the experimental-treatment bog rose to near parity with those in the lagg, resulting in average daily MeHg concentrations and %MeHg levels at bog sites that were equivalent to, or higher than corresponding levels in the lagg (Figure 2.4). Moreover, a disproportionate number of the highest %MeHg levels measured in the experimental treatment were found in samples collected from bog sites rather than lagg sites, as was found in the control treatment (Table 2.1). This finding demonstrates that the ombrotrophic central bog can become an important methylation zone (hot spot) if atmospheric sulfate deposition is sufficiently high.

At current ambient rates of sulfate deposition (~6 kg ha^{-1} yr^{-1}) the lagg margin produces more MeHg than the central bog in S6. However, a 6-fold increase in atmospheric deposition rates sustained over a period of eight years lead to a 5- to 6-fold increase in solid-phase MeHg in the central bog, equivalent to levels measured in the lagg. In S6 the raised central bog represents at least 50% of the total peatland area (conservatively assuming a 15 m wide lagg). Thus the sulfate loading rates used in this study would have effectively doubled the amount of MeHg in this system if applied to the whole wetland. In peatlands with more circular shapes or a larger overall area, the central bog could easily represent an even greater source of MeHg to downstream aquatic systems.

2.5.2 Differential response to experimental sulfate addition

Overall short-term net MeHg production in the experimental treatment was greater than in the control as a result of sulfate additions (Appendix A, Table A4). Nearly 70% of samples collected in the experimental treatment fell above the 90^{th} percentile of %MeHg values measured in the control treatment. However, long-term trends in MeHg production, as inferred from solid-phase %MeHg, indicate that Hg methylation was stimulated in the central bog, but not necessarily in the lagg margin. Hg methylation/demethylation potentials were not measured during this study, but %MeHg in the solid-phase is considered to be an indicator of the long-term balance between *in situ* Hg methylation and demethylation processes (Drott et al., 2008; Tjerngren et al., 2012a). Between 2003 and 2009 solid-phase %MeHg levels in the experimental-treatment bog rose steadily relative to the control treatment bog, whereas no such trend

was observed at lagg sites (Figure 2.4). After eight years of sulfate addition, average solid-phase %MeHg in the experimental-treatment bog was six times greater than in the control bog, while there was no difference in solid-phase %MeHg between the experimental and control lagg.

The lack of a sustained response to sulfate addition in the lagg was somewhat surprising given that the lagg is generally considered to be a better methylating environment than the ombrotrophic central bog. In related studies at other wetlands Branfireun et al. (Branfireun et al., 1996) and Branfireun and Roulet (Branfireun and Roulet, 2002) found much higher porewater MeHg concentrations in localized zones of groundwater discharge as opposed to zones of groundwater recharge. The lagg margin is the dominant hydrologic flow path in the S6 peatland and a discharge zone for water running off both the uplands and the bog mound. Furthermore, the water entering the lagg from the uplands is of slightly higher pH and carries more nutrients, solutes (including sulfate), and labile carbon (Mitchell et al., 2008a; Qualls and Haines, 1992), which might be expected to create a better environment for SRB activity than the nutrient-poor central bog (Minderlein and Blodau, 2010).

A possible explanation for the muted response of the S6 lagg to experimental sulfate addition may be higher demethylation rates experienced in the lagg relative to the bog. Recent work by Kronberg et al. (Kronberg et al., 2012) and Tjerngren et al. (Tjerngren et al., 2012a; Tjerngren et al., 2012b) found elevated demethylation potentials (K_{demeth}) in Swedish alder swamps, which caused these sites to be net sinks for MeHg. It is possible that high demethylation rates in the alder lagg of the S6 peatland prevent the

build up of MeHg in the experimental treatment peat despite elevated sulfate loading. Mineralization of organic carbon is typically greater in the lagg than in the bog as a result of its higher trophic status. In this environment MeHg is a potential electron donor subject to similar degradation and mineralization by bacteria (Gilmour et al., 1998; Pak and Bartha, 1998).

An alternative explanation for the differential response of lagg and bog to experimental sulfate addition is that higher ambient inputs of sulfate from the uplands reduce sulfate-limitation in the lagg, and other reactants limit Hg methylation instead. However, short-term sulfate-limitation of Hg methylation was evident in the lagg as an increase in porewaters %MeHg following spring and summer sulfate applications in 2008 (Figure 2.3). Although these short-term increases in porewater MeHg do not translate into an accumulation of MeHg in lagg peat, elevated Hg burdens were observed in mosquito larvae collected from lagg sites in the experimental treatment relative to those collected from control sites. Newly produced MeHg is generally considered to be more bioavailable than older ambient MeHg owing to complexation of the latter with DOM and/or partitioning to the solid-phase. (Orihel et al., 2008). Therefore biotic-Hg levels suggest that sulfate addition did indeed stimulate production of new MeHg in the lagg of the experimental treatment. Such results suggest that MeHg taken up by the mosquito larvae is less subject to demethylation than porewater MeHg, and if so, biotic-Hg may be a good proxy for methylation rates in systems with high MeHg turnover.

2.5.3 Legacy effects

The finding that experimental sulfate addition did not cause long-term changes in lagg MeHg levels relative to our control may be an artifact of historically elevated sulfate deposition to the surrounding landscape. Wet atmospheric sulfate deposition as measured at the National Atmospheric Deposition Program collection site at MEF has declined steadily since the early 1980s from ~10 kg ha^{-1} yr^{-1} to ~6 kg ha^{-1} yr^{-1} (Coleman Wasik et al., 2012; NADP, 2014). Urban et al. (Urban et al., 1989) reported sulfate fluxes of 1.8-5.2 kg ha^{-1} yr^{-1} from uplands in a similarly sized neighboring watershed between 1981 and 1984 meaning that roughly 20-50% of the sulfate deposited on the uplands at MEF was carried into peatlands by runoff and 50-80% was retained in upland soils. In regions historically affected by much higher rates of atmospheric sulfate deposition, such as the northeastern United States, eastern Canada, and northern Europe, the sulfate deposited to landscapes continues to runoff into aquatic systems, slowing recovery from acidification (Evans et al., 2001; Keller et al., 2003; Mitchell and Likens, 2011; Mitchell et al., 2011; Watmough et al., 2007). The continued release of legacy sulfate from uplands may also be stimulating excess Hg methylation in peatland margins.

2.5.4 Importance of hydrology

The consequences of increased MeHg production in the central bog for downstream aquatic systems will depend primarily on hydrologic connectivity between the bog, lagg, and outflow. In the first year of this study MeHg flux from the S6 peatland as a whole increased by a factor of 1.6 (Jeremiason et al., 2006), although it was impossible to know whether the extra MeHg came from the bog or lagg. The lagg lies

along the dominant hydrologic flowpath in the S6 peatland, and so MeHg produced in the lagg can be directly exported from the system in advective flow. The likelihood of lateral transport of MeHg-rich water from the central bog is probably a function of water table elevation. At high water-table elevations the central bog in S6 contributes substantially to outflow(Mitchell et al., 2008c). As the water table declines, the hydraulic conductivity of the peat and the hydraulic gradient between bog and lagg decrease, slowing the movement of water from the central bog toward the lagg (Gafni and Brooks, 1990).

If the central bog functions like a reservoir for recently produced MeHg then periods of high flow related to hydrological events such as snowmelt or intense storms may cause biogeochemically hot moments (McClain et al., 2003), when stored MeHg is flushed from the system in a pulse. Furthermore, hydrologic fluctuations following drought, which cause a release of sulfate from previously oxidized peat, may stimulate Hg methylation during periods of rising water table elevation resulting in further increases in MeHg flux from the bog. The greater the hydrologic connectivity between the sulfate-impacted central bog and peatland outflow, the greater the possibility that bog-produced MeHg will reach downstream aquatic foodwebs. Alternatively, if demethylation rates in the alder lagg were sufficiently high, MeHg moving slowly from the bog through the lagg could be degraded before reaching the outflow (Kronberg et al., 2012; Tjerngren et al., 2012a; Tjerngren et al., 2012b). In this case the lagg may serve as a MeHg-buffer to downstream aquatic systems.

2.6 Conclusions

The findings in this study highlight the effect of elevated atmospheric sulfate deposition on the spatial dynamics of MeHg production in sulfur-limited peatlands. The narrow lagg margin is generally considered to be the most important zone for Hg methylation in peatlands, particularly where concavities in upland topography focus hydrologic flow and solute inputs (Mitchell et al., 2008b, 2009). However, the primary zone of Hg methylation may expand into the much larger central bog when sulfate loads from atmospheric deposition or drought-induced peat oxidation increase.

In this study experimental sulfate addition appeared to elicit higher levels of MeHg production at bog sites than at lagg sites. While cumulative annual increases in solid-phase MeHg were measured in the experimental-treatment bog, no increases were observed in the experimental lagg. It is possible that high demethylation rates in the alder lagg, observed by others in comparable systems, could lead to the rapid degradation of MeHg produced in response to sulfate addition and thus explain the lack of difference in solid-phase MeHg between control and experimental lagg sites (Kronberg et al., 2012; Tjerngren et al., 2012a; Tjerngren et al., 2012b). This interpretation is supported by porewater and mosquito-larvae data, which suggest that short-term Hg methylation was in fact stimulated in the lagg by experimental sulfate addition. Although the contribution of legacy upland sulfate to current MeHg levels in the lagg margin is unknown, high levels of MeHg observed in the control lagg may also be an artifact of historically elevated sulfate deposition.

Although the potential for MeHg production in the central bog is high under scenarios of elevated sulfate deposition, the importance of MeHg production in the central bog for Hg burdens in downstream aquatic foodwebs essentially comes down to a question of hydrology. As the degree of hydrologic connectivity between the central bog and the peatland outflow increases, so does the likelihood that MeHg flux from the system will increase. One remaining unknown is how quickly MeHg entering the lagg margin from the central bog is demethylated and whether the lagg margin may buffer MeHg-rich runoff from the central bog prior to outflow.

Our findings are consistent with previous research that has identified the lagg margin as the primary zone of Hg methylation in sulfur-limited peatlands. However, the present study also reveals the potential for the central bog to contribute significantly to MeHg flux from peatlands under the right conditions. Because MeHg produced in the central bog appears to accumulate from year to year, elevated rates of atmospheric sulfate deposition can enhance MeHg storage within a peatland. Climatic changes that lead to infrequent but intense rainfall events have the potential to create drying and rewetting cycles that dramatically increase MeHg production in and flux from these systems by providing another source of sulfate to the central bog as well as by increasing hydrologic connectivity between the central bog and peatland outflow.

Table 2.1 Number and percent of samples exceeding the 90th percentile of %MeHg measurements in the control, recovery, and experimental treatments.

Treatment	Year	Total samples	Samples > 90th percentile for treatment	Bog	Lagg
Control	2005-2008	649	65	12 (18%)	53 (82%)
Recovery	2006	84	9	8 (89%)	1 (11%)
Recovery	2007	242	25	17 (68%)	8 (32%)
Recovery	2008	218	22	14 (64%)	8 (36%)
Experimental	2005-2008	256	26	24 (92%)	2 (8%)

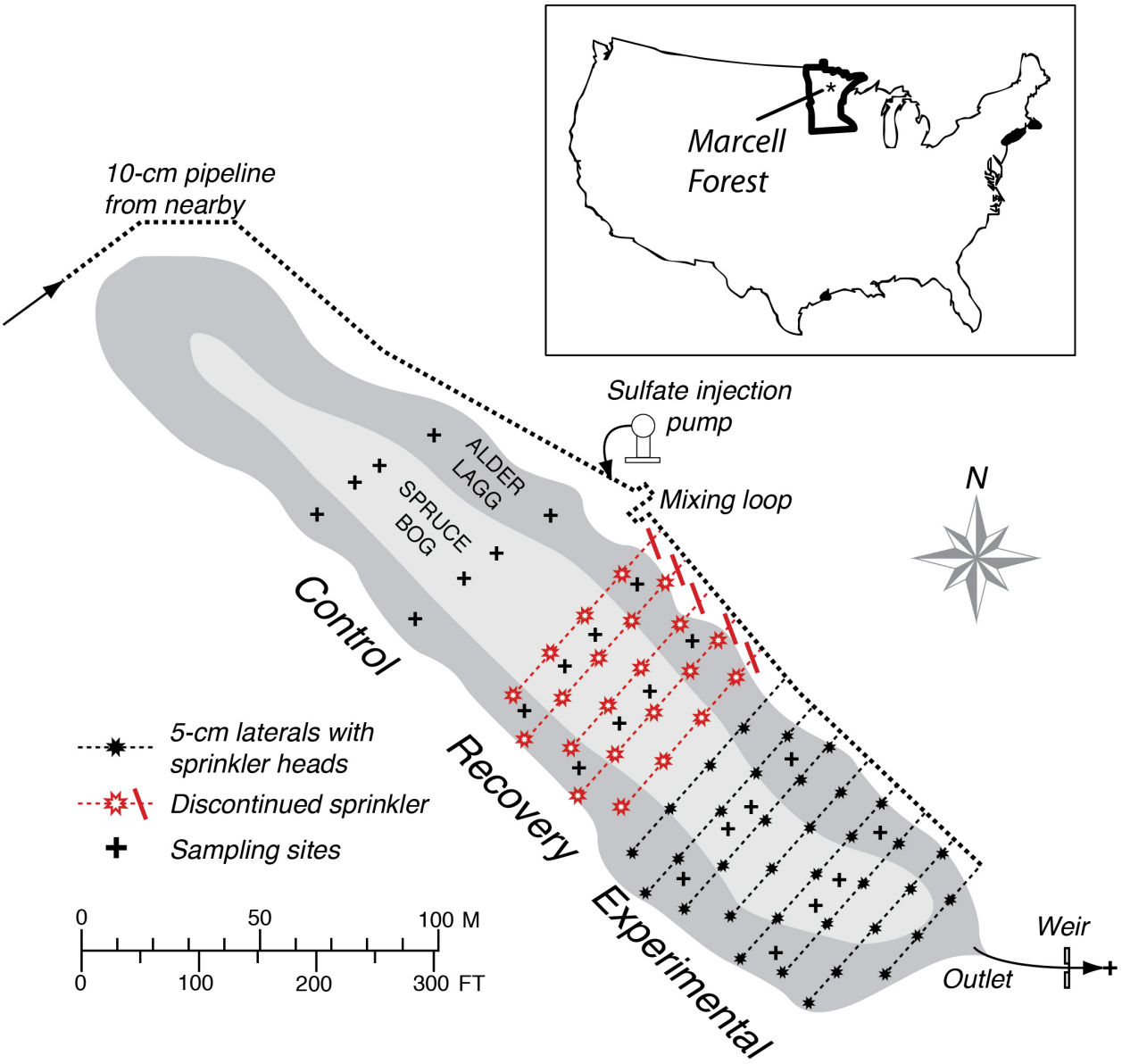

Figure 2.1 Experimental design of the S6 peatland and the locations of porewater sampling sites within each treatment. The inset map shows the location of the Marcell Experimental Forest in northern Minnesota. See text for details.

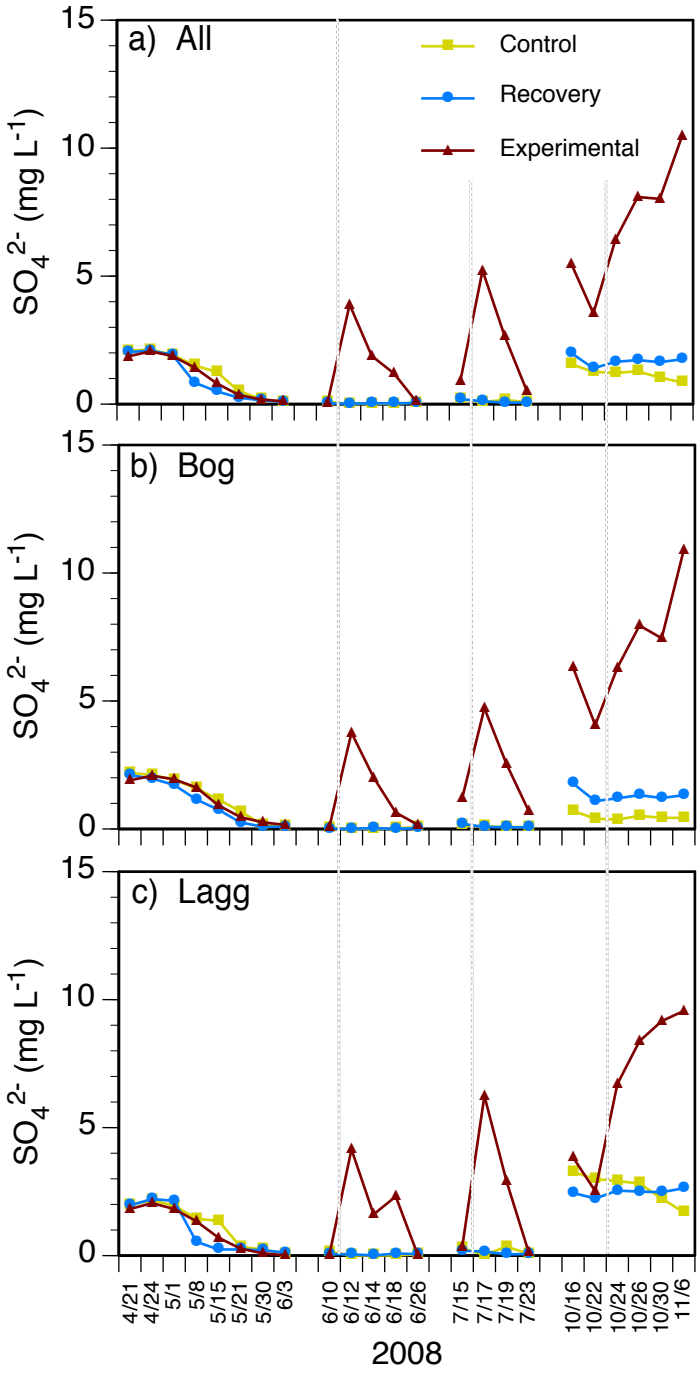

Figure 2.2 Average sulfate concentrations in porewaters from all sites (a), the central bog (b), and the lagg margin (c) of the S6 peatland 2008. Dashed gray lines represent sulfate additions to the experimental treatment.

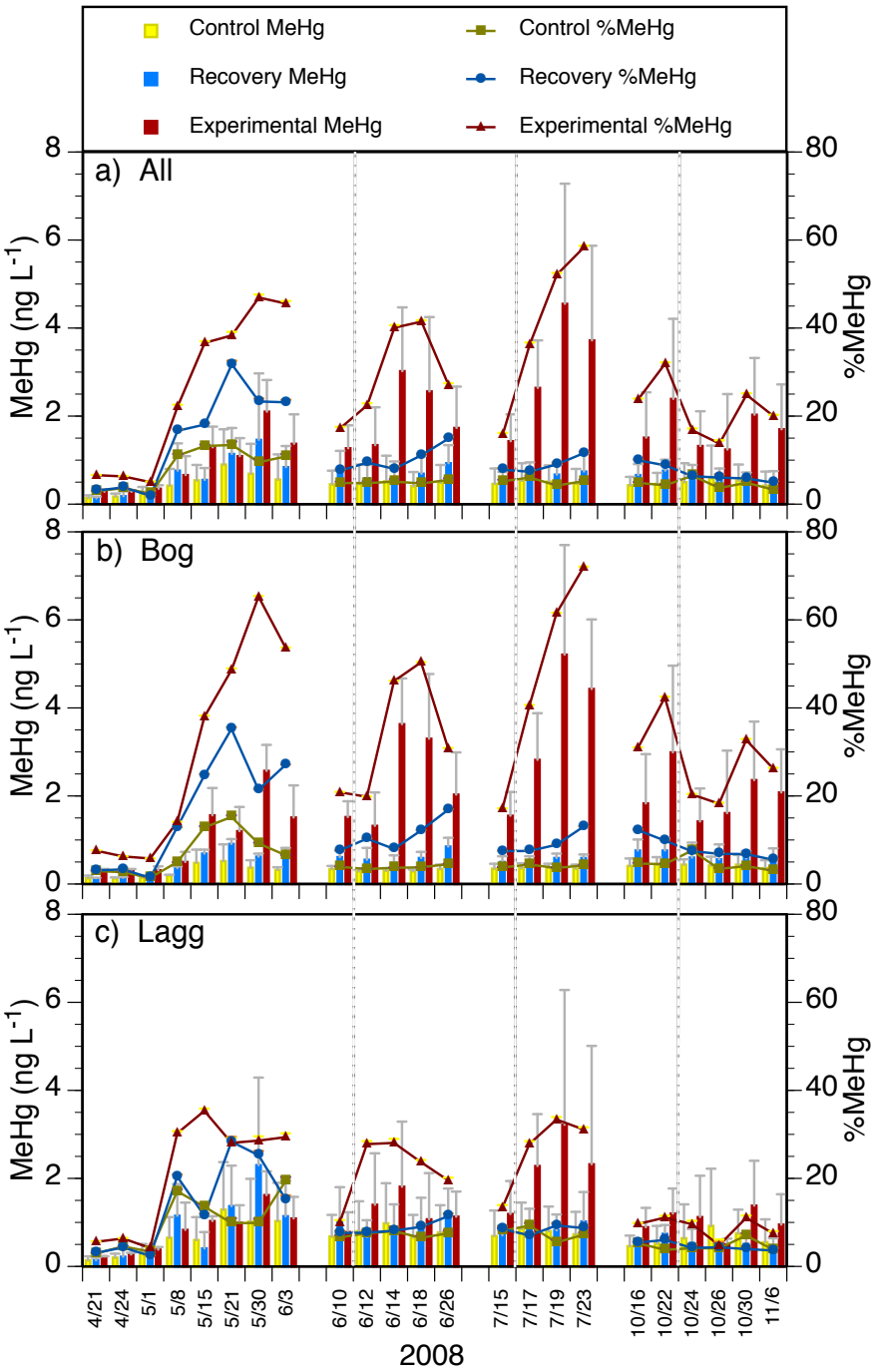

Figure 2.3 Average MeHg concentrations (bars) and %MeHg (lines) in porewaters from all sites (a), the central bog (b), and the lagg margin (c) of the S6 peatland in 2008. Dashed gray lines represent sulfate additions to the experimental treatment.

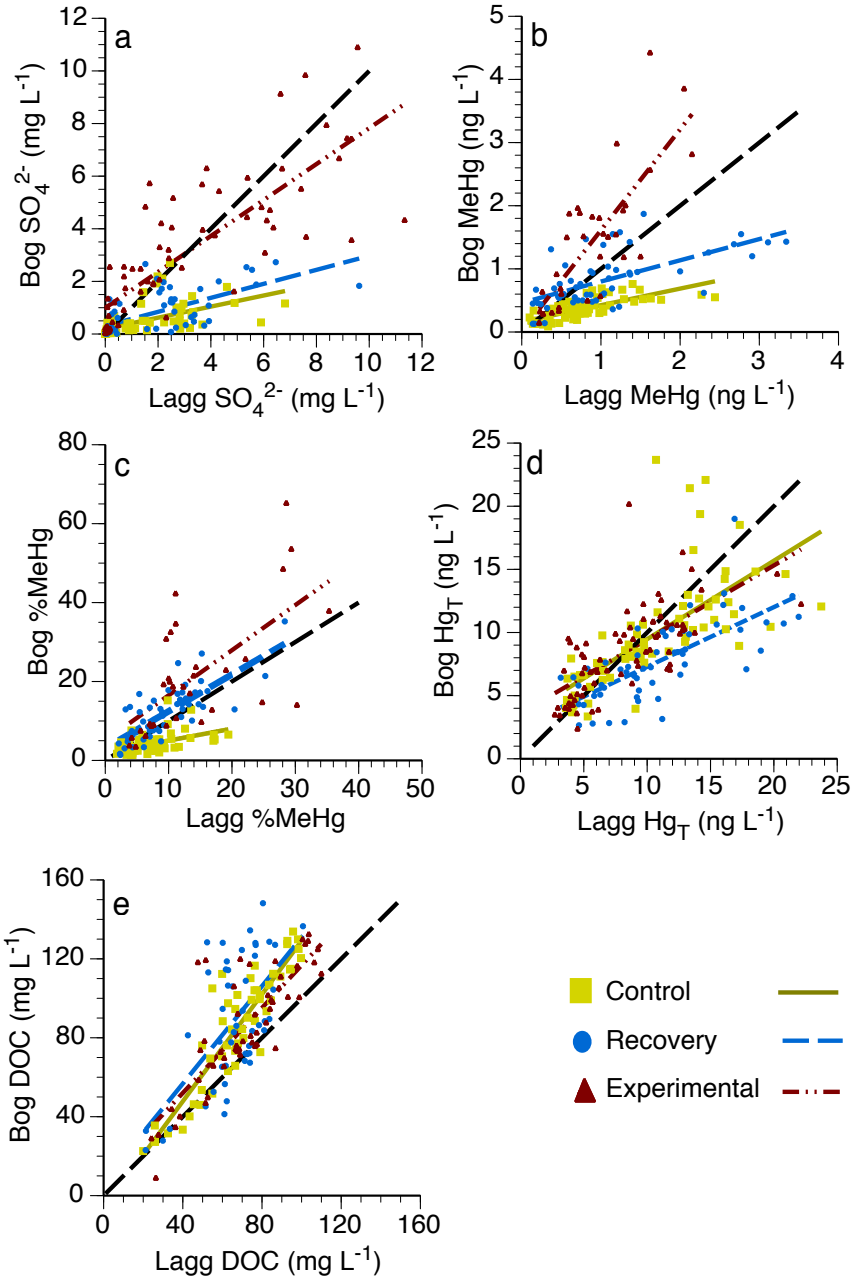

Figure 2.4 Scatterplots of daily average porewater chemistry in bog vs. lagg sites within each treatment. The lines illustrate the overall average difference in porewater chemistry between the bog and lagg and are not meant to indicate a statistical relationship. The dark dashed lines indicate a 1:1 relationship or equivalent porewater chemistry between the bog and lagg.

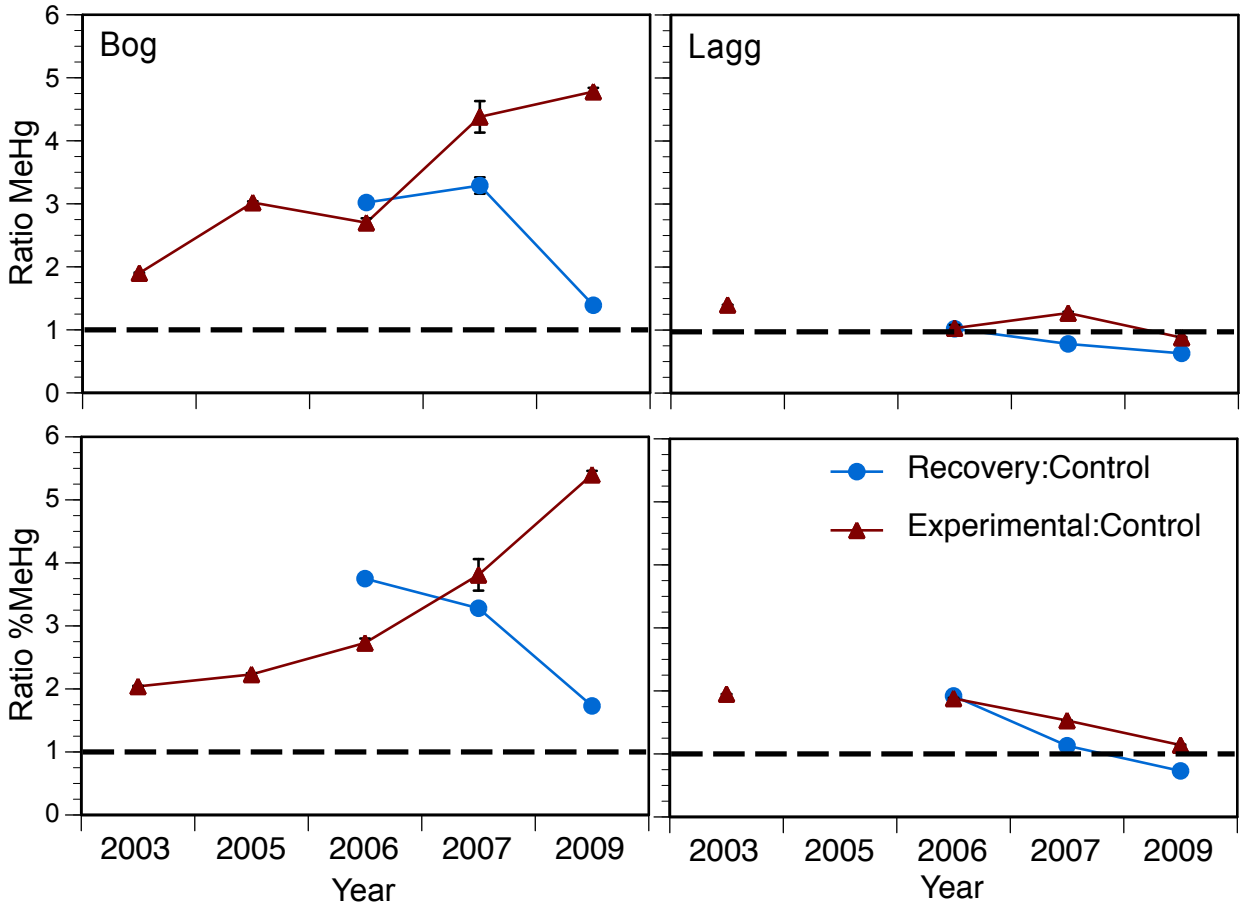

Figure 2.5 Ratios of recovery or experimental treatment MeHg (top) and %MeHg (bottom) in the solid phase to control treatment MeHg and %MeHg in the solid phase in bog sites (left) and lagg sites (right). Dashed horizontal lines depict equivalency with the control treatment.

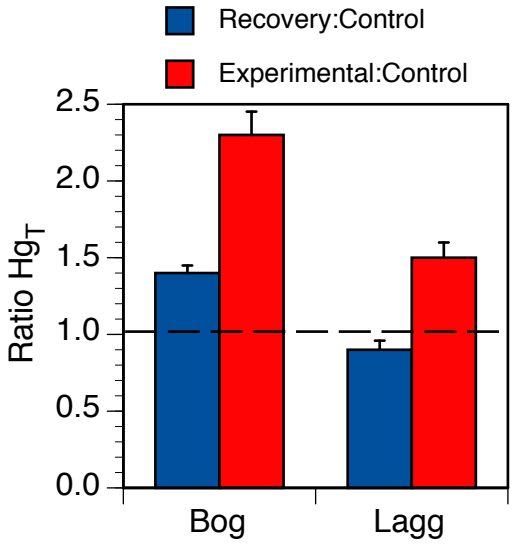

Figure 2.6 Ratio of Hg_T in mosquito larvae from the recovery or experimental treatments to Hg_T levels in the control treatment in bog and lagg sites, spring 2009. Dashed horizontal line depicts equivalency with the control treatment.

Chapter 2

Hydrologic fluctuations and sulfate regeneration increase methylmercury in an experimental peatland

3.1 Summary

A series of severe droughts during the course of a long-term, atmospheric sulfate deposition experiment in a boreal peatland in northern Minnesota created a unique opportunity to study how methylmercury (MeHg) production responds to drying and rewetting events in peatlands under variable levels of sulfate-loading. Peat oxidation during extended dry periods mobilized sulfate, MeHg, and Hg_T to peatland porewaters during rewetting events. Porewater sulfate concentrations were inversely related to antecedent moisture conditions and proportional to past and current levels of atmospheric sulfate deposition. Severe drying events caused oxidative release of MeHg to porewaters and also resulted in increased net MeHg production likely because available sulfate stimulated the activity of sulfate-reducing bacteria, an important group of Hg-methylating bacteria in peatlands. While MeHg concentrations during rewetting events were highest in peat receiving elevated atmospheric sulfate deposition, increases were observed across the peatland. Dissolved Hg_T concentrations also increased in peatland porewaters following drought, but were not affected by sulfate loading and did not appear to be directly controlled by DOC mobilization to peatland porewaters. Peatlands are often

considered to be sinks for sulfate and Hg_T in the landscape and sources of MeHg. Hydrologic fluctuations not only serve to release previously sequestered sulfate and Hg_T from peatlands, but may also increase the strength of peatlands as sources of MeHg to downstream aquatic systems particularly in regions that have experienced elevated levels of atmospheric sulfate deposition.

3.2 Introduction

Peatlands are sites of active biogeochemical cycling for many elements, including sulfur and mercury, because they provide a gradient in oxidation-reduction potentials that are exploited by different microbial communities to gain metabolic energy from chemical transformations (Blodau et al., 2007; Bottrell et al., 2007; Deppe et al., 2010). Peatlands, and wetlands in general, are considered to be sinks for atmospherically deposited sulfate, in part because sulfate-reducing bacterial (SRB) communities consume incoming sulfate (Evans et al., 1997; Pester et al., 2012; Spratt Jr et al., 1987; Urban et al., 1989). However, there is a significant body of literature showing that drought cycles can alter this function and make peatlands sources of sulfate to downstream aquatic environments (Bayley et al., 1986; Dillon et al., 2007; Dillon and LaZerte, 1992; Eimers et al., 2004; Laudon et al., 2004; Mitchell and Likens, 2011; Prechtel et al., 2001; Stoddard et al., 1999). Therefore predicted changes in climate that promote drought conditions (Sheffield and Wood, 2008) could have the secondary effect of recycling sulfate previously sequestered in peatlands and increasing sulfate inputs to downstream aquatic systems.

While sulfate release from peatlands following drought has been widely investigated, little research has been conducted on the response of mercury biogeochemistry to drought and drought-induced sulfate release. Mercury is a contaminant of great concern in many freshwater systems, particularly because the most common organic form of mercury, methylmercury (MeHg), is biomagnified in aquatic food chains putting humans and piscivorous wildlife at risk to its neurotoxic effects (Mergler et al., 2007; Munthe et al., 2007). Peatlands are generally considered to be sinks for total mercury inputs (Hg_T) from atmospheric deposition and upland runoff, but sources of MeHg to downstream aquatic systems (Branfireun et al., 1998; St. Louis et al., 1994). The transformation of inorganic mercury to MeHg in the environment is predominantly an anaerobic process carried out by bacterial communities, particularly SRB. Because mercury methylation can be stimulated by sulfate-addition to sulfur-limited aquatic systems (Branfireun et al., 1999; Gilmour et al., 1992; Jeremiason et al., 2006) drought-induced sulfate release represents a potential mechanism whereby peatlands could become even larger sources of MeHg in the landscape.

Most research investigating the effect of hydrology on mercury cycling has focused on reservoir creation (i.e. inundation/flooding) (Bodaly et al., 1997; Hall et al., 2005; St. Louis et al., 2004), export from watersheds (Babiarz et al., 1998; Balogh et al., 2006; Bushey et al., 2008; Mitchell et al., 2008c), and cascading effects on biota (Bodaly and Fudge, 1999; Bodaly et al., 1984; Evers et al., 2007), but not the direct effects of drought on MeHg production/release within peatlands. Gilmour et al. (Gilmour et al., 2004) performed rewetting incubations on dried Everglades sediments in the laboratory

and observed both sulfate release and a consequent rise in mercury methylation. A few studies have specifically addressed the issue of drought influence on mercury bioaccumulation. Snodgrass et al. (Snodgrass et al., 2000) found that a drying period was important in explaining higher fish mercury levels in South Carolina depressional wetlands, and George and Batzer (George and Batzer, 2008) likewise invoked drought conditions to explain elevated invertebrate mercury levels in the Okeefenokee Swamp.

The study reported here was part of an eight-year whole-ecosystem experiment on the effects of elevated sulfate deposition on MeHg production in a small, boreal peatland (Coleman Wasik et al., 2012; Jeremiason et al., 2006). Two severe droughts occurred during the course of that study, effectively overlaying a drying and rewetting manipulation onto the sulfate addition experiment. The purpose of this paper was to describe the effects of these drought events on mercury cycling in the context of the depositional history of sulfate. The experimental peatland was divided into treatments that received differing sulfate loads, and intensive porewater sampling was used to monitor dissolved sulfate, Hg_T, and MeHg concentrations before, during, and after drought. Solid phase (peat) samples were also collected over the course of the experiment and are discussed in greater detail elsewhere (Coleman Wasik et al., 2012). In addition, water levels were experimentally manipulated in mesocosm enclosures to simulate natural drought-induced changes in sulfur and mercury cycling. Our main objectives were to: 1) determine whether differential atmospheric sulfate loading affected sulfate release following drought; 2) understand how the oxidizing effects of drought

affected mercury cycling; and 3) explore the interaction between drought-induced sulfate release and MeHg production.

3.3 Methods

3.3.1 Site description

The study was conducted in the S6 peatland located within the Marcell Experimental Forest (MEF), a unit of the Chippewa National Forest in northern Minnesota (Figure 3.1). The 2.0-ha S6 peatland occupies an elongate, ice-block depression common in the glacial landscape surrounding the MEF (Sebestyen et al., 2011). The raised ombrotrophic center of the S6 peatland is dominated by an overstory of mature black spruce (*Picea mariana*) and tamarack (*Larex laricina*) and an understory of ericaceous shrubs (e.g. *Chamaedaphne calyculata and Ledum groenlandicum)*, herbaceous forbs (e.g. *Cypripedium acaula and Menyanthes trifoliate)*, and *Sphagnum* spp (MEF, 2013). Alder (*Alnus rugosa*) along the peatland margin delineates the minerotrophic lagg, which receives runoff from a 6.9 ha white spruce (*Picea glauca*) and red pine (*P. resinosa*) upland (MEF, 2013).

The regional climate at the MEF is continental, with annual precipitation averaging 710 mm between 2000 and 2008 (Table 1). A significant portion of the precipitation is received during the winter months, and because hydrology in the S6 peatland is driven by precipitation, spring snowmelt is typically the largest hydrologic event of the year (Nichols and Verry, 2001). The S6 peatland is perched hydrologically above the regional groundwater table, and therefore its water table elevation (WTE) and

outflow are heavily dependent on precipitation. The lagg margin represents the dominant flow-path for both the central bog and upland catchment, with the central bog generally contributing a greater proportion of the total outflow (unpublished data). WTE and outflow are monitored continuously by the USFS Northern Research Station at a centrally located bog well and a 120° V-notch weir, respectively. Upland surface flow and interstitial flow collectors are used to estimate hydrologic inputs from the uplands.

3.3.2 Sulfate deposition experiment

Results reported here were obtained during a long-term study (2001-2008) of the effects of elevated atmospheric sulfate deposition on MeHg production in a sulfur-limited peatland. Ambient sulfate deposition, recorded since 1976 at the MEF (NADP site MN16) (NADP, 2014) has decreased by 50% from 11 kg ha^{-1} yr^{-1} in the early 1980s to approximately 5.5 kg ha^{-1} yr^{-1} in the mid-2000s. Sulfate deposition to the experimental treatment in this study was increased by ~4X the ambient 1990s rate to 32 kg ha^{-1} yr^{-1} in order to simulate late-20^{th} century sulfate deposition rates experienced across much of eastern North America.

The experimental design of the overall study has been described previously (Coleman Wasik et al., 2012; Jeremiason et al., 2006). Briefly, in 2001 the S6 peatland was divided roughly in half into control and experimental treatments, and a PVC rainfall simulator was constructed across the experimental portion. This system consisted of a 10-cm main pipeline that ran along the northern edge of the peatland and was distributed through 13 5-cm lateral lines to rotating sprinkler heads mounted on 1-m vertical risers. Dilute surface water (specific conductivity = 20 µS cm^{-1}) was drawn from a nearby pond,

and a concentrated sodium-sulfate solution was injected into the main pipeline at a point down-gradient of the control treatment. A mixing loop in the main pipeline ensured that the concentrated sulfate was thoroughly mixed with the source water. Sulfate was added in three simulated rainfall events each year (spring, summer, and fall). Each sulfate addition was followed by a rinsing period to wash sulfate off the vegetation, resulting in a total of 6-8 mm of simulated rainfall. In the spring of 2006 a new recovery treatment was established by discontinuing sulfate addition to the up-gradient, one-third of the original experimental treatment. A bromide tracer was added during each application to monitor movement of application water. However, bromide was not conservative in the peat and so served instead as a presence/absence indicator rather than a quantitative tracer.

3.3.3 Water-table mesocosm experiment

A series of 12 water-table mesocosms was installed across the peatland in July of 2007 in order to simulate the effects of natural hydrologic fluctuation on sulfur and mercury cycling. Four 75-cm lengths of 30.5-cm (ID) PVC pipe were driven into homogeneous lawn areas of the central bog within each treatment (control, recovery, experimental). Each mesocosm was allowed to equilibrate overnight, and porewaters were sampled the next day to capture mercury and sulfate concentrations prior to water-table manipulation. Deionized water was then added to each mesocosm until the water table was approximately 1 cm above the peat surface. Not all mesocosm installations were successful in maintaining experimental water levels above the peatland water table. If water levels in mesocosms fell by more than 5 cm overnight (owing to leakage out the bottom), the PVC pipe was repositioned and again monitored for leaks. Mesocosms were

reset up to two times before abandoning the effort at that location. Once mesocosms were successfully installed, porewaters were sampled on days +1, +2, +3, +7, +9, +11, and +13 relative to the water table rise. Deionized water was added periodically to maintain water levels at the peat surface as sampling and evaporative losses caused declines. The mesocosm experiments were initiated one week prior to the summer 2007 sulfate addition. Mesocosms located in the experimental treatment were covered during the sulfate application, following which 130 mg of Na_2SO_4 was added directly to each in a dilute deionized water solution. This application rate was comparable to the amount added to the S6 peatland during the summer sulfate addition.

3.3.4 Porewater sampling

The short-term effects of sulfate addition were monitored through intensive sampling of peatland porewaters before and after each addition. Initially two transects were established in the control and experimental treatments, and four 1-m^2 sampling plots were evenly distributed among the central bog and lagg margins along each transect. In 2006 two transects were established in the newly created recovery treatment and the original experimental treatment transects were relocated further down-gradient to ensure that sampling occurred within the treated area. Instrument sites were also installed in the central bog along the southern transect in each treatment in 2006. A pressure transducer and nested temperature and oxidation-reduction potential probes (at 10-, 20-, and 30-cm depths) interfaced to a Campbell data logger were used to monitor the interaction between local water table elevation and redox conditions in the peat. Porewater samples

were collected in triplicate from bog plots located next to the instrument sites in order to increase sample numbers and assess the local heterogeneity in porewater chemistry.

Porewaters were collected from each plot on days -1, +1, +3, and +7 relative to each sulfate addition as well as on day +14 for every spring and fall addition. Beginning in 2006 porewaters were sampled with greater frequency in the spring, either starting with snowmelt or beginning soon thereafter, and an additional sampling day was added one week prior to the fall sulfate additions. In the fall of 2007 porewaters from each plot were also sampled on days +2, +4, +9, +14, +18, and +27 after a large rainfall event on September 6.

Porewaters were collected using a portable peristaltic pump and a 1.9-cm ID, Teflon probe with a 5-cm perforated tip. The probe was inserted into the peat 5-10 cm below the water-table surface, and porewaters were drawn through 0.64-cm ID Teflon tubing by a peristaltic GeoPump and passed through acid-washed 47-mm Teflon filter holders (Savillex Co.) prefitted with ashed, 0.7-µm, glass-fiber filters into sample bottles. Samples for dissolved Hg_T, MeHg, and major anions were collected from each plot on every sampling day. Samples for dissolved organic carbon (DOC) were collected from each plot one day prior to sulfate additions in 2005 and 2006 and on each sampling day in 2007 and 2008. All mercury samples were collected directly into new, 125-mL PETG bottles using accepted, clean sampling techniques(Bloom and Fitzgerald, 1988) and preserved by acidifying to 0.5% (vol/vol) with high purity HCl. Field duplicates and equipment blanks accounted for 10% of all samples.

3.3.5 Analytical methods

3.3.5.1 Major anions

Porewater samples were analyzed for major anions (SO_4^{2-}, Cl^-, Br^-) by chemically-suppressed, ion chromatography on a Dionex DX-500 according to standard methods. Each run included 10% deionized water blanks, 10% sample duplicates, and check standards. Check standards and duplicates were within 10%, and detection limits for each anion were 0.01 mg L^{-1} in each year.

3.3.5.2 Dissolved organic carbon

Porewater samples were analyzed for DOC according to standard methods by either a UV-persulfate oxidation method on a Tekmar-Dohrmann Phoenix 8000 or by catalytic combustion on a Shimadzu carbon analyzer. All samples were analyzed in duplicate. Check standards and equipment blanks accounted for 10% of analyzed samples. Sample replicates and check standards were within 10% and equipment blanks were generally less than 1 mg L^{-1} DOC each year.

3.3.5.3 *Mercury*

Dissolved Hg_T was analyzed according to EPA method 1631, Revision E. Samples were allowed to oxidize overnight with bromine monochloride to convert all mercury species to Hg^{2+} and then neutralized with hydroxylamine prior to analysis. Mercury was converted to Hg^0 using stannous chloride reduction, purged from solution, and trapped on gold traps. Mercury was then thermally desorbed in a stream of argon and analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS) on a Tekran 2600 Automated Total Mercury Analyzer. The instrument was calibrated daily and each

analytical run included 20% deionized-water blanks, 10% sample duplicates, and 5% matrix spikes. In all years spike recoveries were between 78 and 114%, relative percent differences between duplicates were less than 10%, and method blanks were below 1 ng L^{-1}.

Dissolved MeHg was analyzed according to methods described in Bloom(Bloom, 1989) and Liang et al.(Liang et al., 1994). Samples were first distilled with 8M H_2SO_4 and 20% KCl (wt/vol) in an acid-cleaned, Teflon, extraction manifold. Distillates were refrigerated and analyzed within 48 hours. All mercury species in solution were ethylated using sodium tetraethylborate and then purged from solution in a stream of nitrogen and trapped on Tenax traps. The trapped mercury species were thermally desorbed in a stream of argon or helium and separated during passage through a chromatographic column. The separated mercury species were then converted to Hg^0 in a pyrolytic trap and analyzed by CVAFS on a Tekran 2500 or Brooks Rand Model III spectrometer. The instruments were calibrated daily and each analytical run included 5% deionized-water blanks, 10% sample duplicates, and 5% matrix spikes. In all years spike recoveries were between 98 and 103%, relative percent differences between duplicates were less than 12%, and method blanks were below 0.15 ng L^{-1}.

Poor calibration curve linearity, high blanks, or quality control samples more than 15% deviation from expected concentrations in any Hg_T or MeHg analysis precluded sample analysis until the analytical issue was resolved.

3.3.6 Numerical analyses

All statistical analyses were performed using the statistical software R (R-Development-Core-Team, 2011). The Wilcoxon Signed-Rank analysis was used to compare mean sulfate and mercury concentrations among treatments on each day and within each treatment before and after sulfate additions or storm events. Kruskal-Wallis analyses were used to assess the effect of water-level manipulations and treatment in the experimental water-table mesocosms. A series of regression analyses were performed to ascertain correlation between fluctuations in WTE and sulfate concentrations within each treatment. Sulfate data were averaged by treatment for each sampling day and then log-transformed prior to regression analyses to normalize their distributions. The maximum change in the water-table and the duration of that change were calculated for five different time periods (10-, 20-, 30-, 60-, and 90-days) preceding each porewater sampling date. The position of the water table was incorporated into regression analyses by determining the average WTE over the preceding time period and then calculating the percentile represented by that average based on the entire WTE record (1965-2008).

3.4 Results

3.4.1 Drought in the S6 peatland

3.4.1.1 Effect on water table elevation

The S6 peatland is considered to be a poor fen with little or no connection to the regional groundwater table (Sebestyen et al., 2011). The center of the peatland is raised relative to its margins creating an ombrotrophic system that relies predominantly on

atmospheric precipitation for water and nutrient inputs. The lack of a moderating, regional hydrologic influence results in large inter- and intra-annual variation in water-table elevations (WTE) and outflow (Table 3.1). Water levels and outflow generally reach peak values during and after spring snowmelt, decline over mid- to late-summer, and usually rebound during the fall after vegetation senescence (Figure 3.2). This general pattern varies from year to year. For example, during abnormally wet years there may be no summer decline, while during abnormally dry years there may be no fall rebound (e.g. 1999 and 2006 respectively; Figure 3.2). Severe droughts have occurred at the MEF several times over the nearly 50 years of data collection (1967-68, 1976-77, 1990-91, and 2006-07) and were initiated by a year in which the area received less than 60 cm of precipitation.

The most recent drought occurred during the course of the 8-year sulfate addition experiment in S6 (Figure 3.2). In 2006 the MEF received 56.1 cm of precipitation. The WTE reached its annual maximum of 422.94 m a.s.l. on March 31 during the spring snowmelt and then declined by 1.05 cm day^{-1} until April 15, followed by a slower rate of decline over the rest of the month (0.12 cm day^{-1}). WTE declined by 0.16 cm day^{-1} during May, then by 0.21, 0.48, and 0.36 cm day^{-1} in June, July, and August, respectively. The water table rebounded slightly in late September/early October (rising 0.15 cm day^{-1}), but then resumed a slow decline until snowmelt the following spring.

In February 2007 the WTE in S6 reached 422.28 m a.s.l. – the lowest level measured in 30 years – and then rebounded more than 55 cm during the snowmelt period in late April, resaturating peat that had been dry for nearly 9 months. The S6 WTE

remained relatively stable throughout May and then began a decline through the summer, similar to that seen the previous year. WTE declined by 0.35, 0.47, and 0.29 cm day^{-1} in June, July, and August, respectively. In September several large rain events over the MEF raised the WTE 39 cm over the course of 6 weeks (Sept. 6 - Oct 19). The water table began another decline in late October that lasted through the winter. However, the wetland froze in a saturated condition as opposed to the very desiccated state of the previous year. In 2008 the WTE resumed a more typical pattern as described previously.

3.4.1.2 Effect on oxidation-reduction potential

The oxidation-reduction potentials measured within each treatment at three different depths in 2006, 2007, and 2008 provided insight on the depth penetration of oxygen into the peat as water tables rose and fell (Figure 3.3). Generally redox conditions were moderately elevated in the early spring of each year and then became more negative as the peatland thawed and warmed. As the water table fell past each probe depth during the summer the corresponding redox potentials jumped to very positive values indicating the intrusion of oxygen. When the water table rebounded in the fall redox potentials declined slowly toward their previous levels, presumably as oxygen was consumed.

The peat at 10 cm in each treatment was largely subject to oxidizing conditions regardless of whether the peatland was experiencing drought or not (Figure 3.3). Negative redox values were observed only during the spring when the water table was at or near the peat surface. The peat at 20-cm depth experienced larger changes in redox conditions over the course of each year in response to declining water tables and large

rainfall events. Strongly negative values prevailed during the spring and early summer periods while the late summer and fall were characterized by positive redox values. Large rainfall events on July 1, 2007 and July 13, 2008 caused transient increases in redox values at 20-cm depth (Figure 3.3 d-i), possibly owing to downward percolation of oxygenated rainwater. Shortly thereafter the WTE continued its steep summer decline, and redox potentials spiked upward and remained there well into the fall of both years. Redox conditions were most consistent at 30-cm depth among treatments and years, declining to low steady values in spring or early summer and then spiking upward as WTE fell below the probe depth in mid-summer. Because water tables fell particularly low in 2006 and 2007, oxygen was able to penetrate to 30-cm depth for extended periods of time (Figure 3.3 a-f).

3.4.2 Response of Porewater Sulfate and Mercury to Drying Events

3.4.2.1 Water table elevation and sulfate addition

Sulfate was added to the experimental treatment three times during each field season between 2002 and 2008 with the goal of stimulating mercury methylation. The effectiveness of each sulfate addition was influenced by the position of the water table, as exemplified by trends in porewater %MeHg and sulfate concentrations in 2005 (Figure 3.4). Over the sulfate addition and sampling period in spring of 2005 the WTE was high, averaging 422.825 m a.s.l. Sulfate concentrations in experimental treatment porewaters increased nearly two orders of magnitude from near detection before the sulfate addition to 1.92 mg L^{-1} after the addition. Three days after the sulfate addition, as sulfate concentrations were declining, %MeHg rose by 3X (from 12% to 37%). Because

concurrent Hg_T concentrations remained constant in the experimental treatment, this MeHg rise is attributed to increased production. Sulfate and Hg_T concentrations and %MeHg in the control treatment were stable (<0.14 mg SO_4^{2-} L^{-1}, <5 ng Hg_T L^{-1}, and 5-8% MeHg) throughout the spring period.

By the time of the summer sulfate addition water tables had fallen 13 cm since the spring addition, and in contrast to the earlier period, sulfate concentrations did not increase in the experimental treatment, but instead remained similar to control treatment levels, likely because added sulfate did not reach the water table. Experimental treatment %MeHg levels also remained stable over the period, but were elevated 2-3 times control treatment levels. Again Hg_T concentrations in the control and experimental treatments were stable and consistently low over the monitoring period (4-5 ng L^{-1}; Figure 3.4). DOC levels during the summer application period were 50% higher than spring concentrations.

Sulfate concentrations were already elevated in both control and experimental treatment porewaters (0.86 and 3.17 mg L^{-1}, respectively; Figure 3.4) prior to the fall-2005 sulfate addition, which itself was preceded by an 8-cm rain event. Sulfate concentrations in experimental treatment porewaters increased to 5.67 mg L^{-1} following the addition, while %MeHg increased only modestly (from 11.0% to 15.4%), despite sulfate concentrations that were nearly three times those associated with a 3X increase in %MeHg after the spring addition. Moreover, %MeHg levels in the control treatment were stable over the sampling period and lower than during either the spring or summer sulfate additions (2.7-3.5%). Hg_T concentrations in both treatments were 3-4 times higher than

at any time during the previous spring or summer (Figure 3.4), and DOC concentrations were twice spring concentrations.

3.4.2.2 Rewetting events

The severe droughts in 2006 and 2007 and the rewetting events that followed caused large swings in WTE and highlighted the effects of hydrologic fluctuations on sulfur and mercury biogeochemistry in the S6 peatland.

3.4.2.2.1 Spring thaw period

The 2006 drought persisted into the winter causing the S6 peatland to freeze in an oxidized state. Therefore an extensive sampling campaign was undertaken in the spring of 2007 to monitor sulfur and mercury cycling as the peatland resaturated. On March 26 pooled snowmelt was sampled from the frozen peat surface and water chemistries were found to be uniform among treatments (2-3 mg SO_4^{2-} L^{-1}, 4-8 ng Hg_T L^{-1}, 0.14-0.18 ng MeHg L^{-1}, and 1.7-3.9 % MeHg; Figure 3.5). As the peat slowly thawed over the next 6 weeks a "natural" sulfate addition ensued. Sulfate concentrations peaked at very high levels for this peatland (3.04, 5.72, and 7.89 mg SO_4^{2-} $L^{-}1$ in the control, recovery, and experimental treatments, respectively). As sulfate concentrations declined MeHg concentrations and %MeHg reached peak levels that were significantly higher than early season lows ($p<0.05$) and were significantly different among treatments ($p<0.05$; control = 1.18 ng MeHg L^{-1}, 10%; recovery = 2.06 ng MeHg L^{-1}, 16%; and experimental = 2.60 ng MeHg L^{-1}, 25%). Hg_T concentrations increased significantly in the control and recovery treatments to 9-16 ng L^{-1}, respectively, ($p<0.05$) and more than doubled relative to levels observed during the first sampling in each treatment. However, Hg_T

concentrations did not show any systematic differences among treatments over the monitoring period. DOC concentrations rose steadily over the entire spring thaw period and were not significantly different among treatments ($p > 0.05$).

The sampling schedule developed for the spring of 2007 was followed in the spring of 2008 because antecedent moisture conditions in the spring of 2008 (described above) were opposite those in the spring of 2007 and provided a natural, experimental comparison (Figure 3.5). Sulfate concentrations were again near 2 mg L^{-1} in snowmelt water pooled on the frozen peat surface in all three treatments. However, in 2008 sulfate concentrations remained nearly identical among treatments over the entire sampling period and concentrations declined steadily over the thaw period to near detection limits just prior to the spring 2008 sulfate addition. Despite much lower sulfate concentrations during the spring thaw period, MeHg concentrations followed a similar pattern to that observed in 2007 (Figure 3.5). Peak MeHg concentrations were somewhat lower than those seen in 2007 (0.9, 1.46, and 2.1 ng L^{-1} in the control, recovery, and experimental treatments, respectively), but %MeHg levels appeared to be higher in 2008 and the difference between the control treatment and the recovery and experimental treatments was more pronounced than in 2007 (Figure 3.5). Hg_T concentrations were generally lower than in 2007 again there were no significant differences in Hg_T concentrations among treatments ($p>0.05$). Dissolved organic carbon concentrations rose steadily again over the entire 2008 spring thaw period and were not significantly different among treatments ($p > 0.05$).

3.4.2.2.2 Fall water table rise

In September 2007 a series of large rainfall events drove a relatively rapid water-table rise and relieved the severe summer drought. As was seen during the rewetting event in the spring of 2007, sulfate concentrations rose significantly from late-July values as the peat resaturated (Figure 3.6; $p < 0.003$), and significant differences existed in peak sulfate concentrations among the treatments ($p<0.05$; 3.10 mg SO_4^{2-} L^{-1}, 3.98 mg SO_4^{2-} L^{-1}, and 7.78 mg SO_4^{2-} L^{-1}, in the control, recovery, and experimental treatments, respectively). In early September following the first rainfall event, average MeHg concentrations and %MeHg in the control and recovery treatments were comparable with late July values (Figure 3.6), while in the experimental treatment MeHg concentrations were significantly lower ($p < 0.02$). Subsequently and over the course of three additional rain events, MeHg concentrations and %MeHg rose significantly ($p < 0.05$), reaching peak levels by early October. Hg_T concentrations were also significantly lower in early September relative to late July ($p<0.008$), and then rose significantly by late September (10-13 ng L^{-1}; $p<0.001$) and were similar among treatments throughout the entire water-table rise. Fall DOC concentrations were comparable to late-July levels and remained relatively constant over the entire monitoring period.

3.4.3 Experimental water table manipulation

In mid-July of 2007 a series of water-table mesocosms was installed across the S6 peatland, and WTE was experimentally raised. Hg_T, MeHg, sulfate, and DOC concentrations in pore waters were measured one day prior to the WTE rise and for up to two weeks thereafter. The effects of the water table experiments varied by treatment and by chemical constituent (Figure 3.7). In the control and recovery treatments the water

table rise did not have a significant effect on any of the chemical constituents measured ($p > 0.05$; Kruskall Wallis), whereas in the experimental treatment the water table rise significantly affected all measured constituents ($p \leq 0.05$). Hg_T concentrations rose over the duration of the experiment while DOC concentrations fell. Sulfate, MeHg, and %MeHg each peaked two days after the water table rise and then declined until the sulfate addition on day-8. Sulfate concentrations peaked again on day-9 after sulfate was added to the experimental treatment, while MeHg and %MeHg peaked on day-11.

3.5 Discussion

3.5.1 Sulfate release after drought

3.5.1.1 *Sulfate and antecedent moisture conditions*

The sulfate concentrations measured in S6 porewaters were similar to those reported for other boreal peatlands (Mitchell et al., 2008b; St. Louis et al., 1994) as well as for peatland mesocosms experimentally amended with sulfate (Bergman et al., 2012; Branfireun et al., 1999). However, the sulfate concentrations in this study tended to be much lower than those measured in areas that are currently, or were historically, impacted by high levels of atmospheric sulfate deposition, such as the northeastern United States (Mitchell and Likens, 2011; Selvendiran et al., 2008) and eastern Canada (Eimers and Dillon, 2002; Eimers et al., 2007; Warren et al., 2001). Sulfate concentrations in S6 porewaters rose following each extended dry period in this eight-year study, which is consistent with observations in other peatland, temperate wetland, and stream systems (Bayley et al., 1986; Bayley et al., 1992; Devito and Hill, 1999;

Eimers and Dillon, 2002; Eimers et al., 2007; Kerr et al., 2012; Mitchell and Likens, 2011; Warren et al., 2001).

In this study the average sulfate concentration for each sampling date (excluding experimental treatment values immediately following sulfate addition) appeared to be inversely related to antecedent moisture conditions. Porewater sulfate concentrations were lowest when the water table had been high over the preceding time period and were highest when the water table had been low. Furthermore, the relationship between porewater sulfate and changes in WTE became stronger with increasing length of the drawdown period as indicated by the higher r^2 and lower p-values for the 60- and 90-day WTE regressions as compared to the 10-day WTE regressions (Table 2). Apparently, the longer the drought, the greater the oxidation of the peat. The increasing strength of the relationship between sulfate concentrations and the of length of the drawdown period is not surprising given that other studies have found that the sulfate that appears during a rewetting event comes from the oxidation of organic sulfur compounds stored in the peat (Devito, 1999; Mandernack et al., 2000; Mörth et al., 1999). Isotopic studies of sulfur cycling in peat have found that sulfate added to peatland mesocosms is predominantly incorporated into the organic-sulfur fraction of the peat matrix through bacterial sulfate reduction and plant uptake (Bartlett et al., 2009; Chapman and Davidson, 2001), and that the sulfate released during rewetting events has a light isotopic signature relative to atmospheric deposition, suggesting reoxidation of sulfur from the "lighter" carbon-bound sulfur pool (Mandernack et al., 2000; Mörth et al., 1999).

The precipitation-driven hydrology of the S6 peatland allowed water tables to decline as much as 50 cm in particularly dry years, causing desiccation and oxidation of deep peat layers that normally experience strongly reducing conditions (Figure 3.3). Dramatic hydrologic fluctuations coupled with the high organic content of the peat make it likely that the sulfate released during rewetting events in this peatland comes from the carbon-bound sulfur pool. Furthermore, inorganic sulfur concentrations were low across the peatland (3 ± 2%) making readily oxidized sulfur compounds like AVS an unlikely source of recycled sulfate.

3.5.1.2 Sulfate release after elevated sulfate deposition

For any given drying event more sulfate was mobilized into porewaters in the experimental treatment than in either the control or recovery treatments. Following rewetting events in the spring and fall of 2007, sulfate concentrations in experimental-treatment porewaters were more than twice that in the control treatment, while sulfate concentrations in the recovery treatment were intermediate between the control and experimental treatments (Figures 3.5 and 3.6). Because sulfate disappeared from porewaters following sulfate additions and rewetting events, and because no significant differences were found in the solid total-sulfur pool among the treatments(Coleman Wasik et al., 2012), it appears that a greater fraction of the organic sulfur pool was available for release in peat that had recently experienced elevated sulfate loading. Furthermore, the finding that sulfate release was greater in the recovery treatment than in the control treatment two years after sulfate additions had ended indicates that this more labile organic sulfur pool persisted for some time after elevated sulfate deposition had

ceased. These observations provide support for our previous hypothesis (Coleman Wasik et al., 2012) that newly added sulfate gradually becomes incorporated into more recalcitrant forms of organic sulfur over time.

The water-table mesocosm experiments confirmed both the differential remobilization of sulfate among treatments and the importance of the duration of WTE drawdown and peat oxidation. Mesocosms in the experimental treatment experienced a significant increase in sulfate concentrations following the water table manipulation (increased WTE). No such sulfate rise was detected in the control or recovery treatments, and the rise that did occur in the experimental treatment was much lower than that observed following the 2006 and 2007 droughts. Average peak sulfate concentrations in the experimental treatment following each drought were roughly 8 mg L^{-1} as compared to 1.0 mg L^{-1} in experimental-treatment mesocosms following the WTE manipulation. The muted release in the mesocosms was likely a result of the short oxidation period experienced by the peat prior to mesocosm installation. The peat was not as desiccated as it had been during the 2006 and 2007 droughts—only the top 10-15 cm of peat experienced oxidizing conditions for approximately 3-4 weeks. Shorter duration drawdowns likely affect loosely-bound sulfate and labile organic sulfur compounds, whereas during extended droughts microbial communities and physical processes may begin to break down more recalcitrant pools of organic sulfur leading to greater sulfate remobilization.

The finding that sulfate is remobilized from wetlands following drought is not unique to this study (Bayley et al., 1986; Devito and Hill, 1999; Eimers and Dillon, 2002;

Eimers et al., 2007; Kerr et al., 2012; Mitchell and Likens, 2011; Warren et al., 2001). However, most previous research has involved ecosystems that were experiencing concurrent changes in ambient sulfate deposition and regional hydrology (drought cycles). The experimental design of the study presented here elucidates the additive effect of past and current sulfate deposition levels on the naturally occurring release of sulfate caused by drought cycles and provides insight into the mechanisms whereby sulfate release from historically impacted peatlands may decline.

3.5.2 Effect of drought on mercury cycling

3.5.2.1 Total mercury

Total mercury (Hg_T) concentrations in S6 porewaters averaged between 3 and 12 ng L^{-1} during most sampling periods, which is similar to values reported for other peatlands (Heyes et al., 2000; Mitchell et al., 2008b; Regnell and Hammar, 2004; Selvendiran et al., 2008). However, during the fall of 2005 and the spring and fall of 2007, average Hg_T concentrations in porewaters jumped to 12-20 ng L^{-1} (Figures 3.4-3.6). These three sampling periods coincided with rewetting events in S6, likely indicating oxidative release of Hg_T from peat. The spring of 2007 and 2008 present a natural experimental contrast between dry and wet antecedent moisture conditions and its effect on Hg_T release. Over the prolonged snowmelt period in 2007 (Mar 26 - Apr 25) average Hg_T concentrations were 49-77% higher than during the hydrologically similar period in 2008 (Apr 21 - May 1). Over the entire spring thaw period in 2007 (Mar 26 - May 16) average Hg_T concentrations were 109-142% higher than the hydrologically similar period in 2008 (Apr 21 - May 1). It is interesting to note that, Hg_T and sulfate

release were very different following water table rise in the fall of 2007. Whereas sulfate concentrations 2 days after the initial fall 2007 water table rise were an order of magnitude higher than they had been on the last sampling day of the summer addition, Hg_T concentrations were 20-50% lower than they had been on the last sampling day of the summer addition. Furthermore, Hg_T concentrations remained stable for more than a week after the first major rain event that initiated the water table rise. Once Hg_T concentrations did start to rise, they more than tripled over the following four weeks. These observations suggest that peatlands have the potential to become large sources of mercury to downstream systems if mercury binding within the peat is disrupted by drought-induced oxidation.

The observed Hg_T release was not controlled by DOC. Given the close association between mercury and organic matter (Dittman and Driscoll, 2009; Driscoll et al., 1995; Kolka et al., 2001), it might be expected that the amount of Hg_T released would remain stable relative to DOC during peat oxidation and resaturation following a drought. However, we found that Hg_T concentrations in porewaters were substantially elevated relative to DOC one month following rewetting events in the fall of 2005 and 2007, indicating that in the short-term release of Hg_T following drought is more pronounced than that of DOC. It has been proposed that DOC may be an appropriate proximal measure for continuous monitoring of Hg_T export from watersheds (Dittman and Driscoll, 2009). However, the finding that Hg_T and DOC releases from peatlands respond differently to severe drought means that care should be taken when extrapolating mercury export from watersheds using DOC measurements alone.

Sulfate additions did not appear to affect porewater or solid-phase Hg_T concentrations during wet or dry periods, contrary to observations of Akerblom et al. (Åkerblom et al., 2013) who found that long-term sulfate addition (10-20 kg ha^{-1} yr^{-1} for 14 years) to peatland mesocosms caused declines in solid phase Hg_T. In our study the inventory of Hg_T in the top 8 cm of peat in the experimental treatment was generally lower than that in the control treatment each year (with the exception of 2005), although the differences were not significant, nor was there a trend in the experimental treatment over the course of the eight-year study (Coleman Wasik et al., 2012). Sulfate addition might have been expected to mobilize mercury from the peat if that mercury was released from the carbon utilized by sulfate-reducing bacteria (SRB) or if sulfides generated by SRB activity caused mercury to be stripped from the solid phase. There was no evidence of this, as Hg_T concentrations in the control treatment porewaters were generally higher than those in the experimental and recovery treatments on a given sampling day, and there was no systematic trend in porewater Hg_T in the recovery treatment that would otherwise indicate a lingering effect of previous sulfate additions. Perhaps no effect was observed because the large pool of mercury present on the solid phase was a more important control on porewater Hg_T concentrations than the enhancement of microbial activity due to sulfate addition (Coleman Wasik et al., 2012).

3.5.2.2 Methylmercury

MeHg concentrations and %MeHg observed in this study (0.1 – 4.0 ng L^{-1} and 2 - 50%, respectively) fall within the ranges reported in other boreal peatland studies (Bergman et al., 2012; Branfireun et al., 1999; Heyes et al., 2000; Mitchell et al., 2008b).

The MeHg present in peatland porewaters can come either from physical release (desorption) from the solid phase (where >99% of MeHg is found) or from net methylation. Mercury methylation requires bioavailable inorganic mercury and a carbon source, and may be stimulated by excess sulfate (Benoit et al., 2002). Because MeHg and the substrates required for mercury methylation can all be released from the solid phase through peat oxidation it is difficult to know whether simple oxidation or stimulated methylation is more important in controlling MeHg flux from wetlands following drought. In this study both mechanisms (release and production) were observed to occur.

As described above, sulfate concentrations rose dramatically in all treatments in the spring of 2007 as the S6 peatland resaturated after a nine-month drought. Given that spring sulfate additions during the entire eight-year study consistently induced large methylation events in the experimental treatment (Coleman Wasik et al., 2012; Jeremiason et al., 2006), we expected that this large drought-induced pulse of sulfate in peatland porewaters would have a similar effect on MeHg production across treatments. Indeed, average porewater MeHg concentrations were significantly higher (29%, 80%, and 149% in the control, recovery, and experimental treatments, respectively; $p<0.01$, Kruskall Wallis) during the snowmelt period in 2007 (Mar 26 - Apr 25) than in the hydrologically similar period in 2008 (April 15th to May 1st). On the other hand, %MeHg levels during snowmelt were statistically the same between the two years ($p = 0.54$; Kruskall-Wallis), suggesting that release of MeHg (and Hg_T) from the solid phase occurred as the peat was resaturated following drought. However, as sulfate

concentrations began to decline, MeHg concentrations and %MeHg levels rose further, while Hg_T concentrations remained relatively stable, likely indicating new MeHg production as a result of SRB activity.

Despite significantly higher MeHg concentrations in the spring of 2007 as compared with 2008 ($p < 0.01$; Kruskall-Wallis), %MeHg levels in 2007 were significantly lower than in 2008 ($p < 0.005$; Kruskall-Wallis). That is, a larger fraction of porewater Hg_T was methylated in 2008 relative to 2007. This difference may be a function of the stable hydrologic conditions (consistently high WTE) present during the spring of 2008 as opposed to the spring of 2007 (initially low WTE). Because SRB activity requires anoxia, sulfate reduction and Hg methylation may have been inhibited for a period of time in 2007 by elevated oxygen in the peat profile. This idea is supported by the observation that sulfate concentrations continued to increase beyond the initial mercury release in late April of 2007. It is less likely that this delayed effect was a result of temperature because in each spring sulfate concentrations began to decline well before the peat had thawed completely (unpublished data).

The fall rewetting event in 2007 provided further confirmation that drought can cause not only MeHg release, but also stimulate MeHg production. The largest rise in Hg_T concentrations occurred between September 20th and September 24th, and thereafter Hg_T concentrations stabilized. On the other hand MeHg concentrations and %MeHg levels in the recovery and experimental treatments continued to increase beyond September 24th coincident with declining sulfate concentrations. These sustained

increases likely represent new MeHg production caused by the drought-induced sulfate pulse.

This study allowed us to observe the effect of different atmospheric sulfate deposition rates on MeHg release and production in the context of hydrologic variability. More MeHg was produced and released in experimental and recovery treatments than in the control treatment following each drought. We previously reported (Coleman Wasik et al., 2012) much higher MeHg concentrations in the solid phase within the experimental and recovery treatments relative to the control treatment, and suggest here that a larger pool of MeHg is available for drought-induced release in peat that has experienced elevated rates of sulfate deposition. Furthermore, because the organic sulfur pool formed from recent sulfate deposition is more susceptible to oxidation and mobilization following drought, the potential exists for greater MeHg production from the activity of SRB as peat is resaturated. Finally it appears that recent exposure to elevated sulfate deposition may have "primed" SRB communities in the experimental and recovery treatments. In the spring of 2008 sulfate concentrations in peatland porewaters were the same among treatments after snowmelt and over the entire spring thaw period. However, MeHg concentrations and %MeHg levels increased to a much greater degree in experimental and recovery treatments relative to the control treatment. The observation that greater methylation ensued in treatments exposed to elevated rates of sulfate deposition – despite having, for a period of time, similar concentrations of porewater sulfate – may indicate that the bacterial community in treated peat was more able to efficiently reduce added sulfate and as a result methylate more mercury.

3.6 Conclusions

This study provides important insights on the effects of drought and subsequent water table fluctuations on sulfur and mercury cycling in a boreal peatland. Because two severe droughts occurred during the course of an experimental manipulation of atmospheric sulfate deposition, we were able to examine the *in situ* interaction of hydrologic fluctuations with varying sulfate loads on sulfur and mercury biogeochemistry. Sulfate concentrations in peatland porewaters were a function of antecedent moisture conditions in combination with experimental manipulations. Because the sulfate that reappeared in porewaters during rewetting events likely came from the large pool of organic sulfur in the peatland, prolonged water table drawdowns lead to greater sulfate release in all treatments. However, sulfate mobilization was highest and most responsive to drying conditions in the experimental treatment where recently added sulfate had become incorporated into the organic sulfur pool, yet was still relatively labile compared with organic sulfur in the control treatment.

The effect of antecedent moisture conditions on mercury biogeochemistry was more complicated. Although Hg_T concentrations increased significantly in peatland porewaters during rewetting events following drought, Hg_T release was not always immediate. Despite the common finding that peatlands are sinks for Hg_T in the landscape, the large release of mercury from the peat following drought provides evidence that peatlands can also be sources of inorganic mercury to downstream aquatic systems under certain hydrologic conditions.

In contrast, wetlands are well-known sources of MeHg to downstream aquatic systems (Babiarz et al., 1998; Bushey et al., 2008; St. Louis et al., 1994), and sulfate stimulation of *in situ* methylation has almost certainly contributed to the flux of MeHg from the S6 peatland (Jeremiason et al., 2006). Based on findings from the full eight years of sulfate addition (Coleman Wasik et al., 2012), it was expected that the high porewater sulfate observed following the 2006 and 2007 droughts would significantly stimulate mercury methylation in peatland porewaters. Although there was evidence of increased MeHg production as the drought-induced sulfate was consumed, our results also demonstrate the potential for drought to further elevate MeHg flux from peatlands because of oxidation and desorption of MeHg from the solid phase.

This study was equally revealing regarding the effects of elevated sulfate deposition on mercury biogeochemistry beyond stimulation of mercury methylation. Although mercury export from the S6 peatland was not examined in this study, peatland porewaters represent an important component of outflow from this system under the right hydrological conditions (Mitchell et al., 2008c). In our experimental treatment, sulfate release following drought was greater than that in the control treatment. Not only was that sulfate then available to drive SRB activity and Hg methylation, but it was also available for export to downstream aquatic systems (e.g. lakes and other wetlands) that could be equally susceptible to *in situ* net methylation. Drought-induced MeHg release in the experimental treatment was also greater relative to the control treatment during rewetting events because a larger pool of MeHg had built up in the solid phase of the experimental treatment as a result of chronically elevated sulfate loading (Coleman

Wasik et al., 2012). This observation implies the potential for greater MeHg export from sulfate-impacted peatlands to downstream aquatic systems relative to unimpacted peatlands. Finally the observation in the spring of 2008 that net methylation (as inferred from changes in %MeHg) was greater in the experimental treatment relative to the control – despite similar concentrations of porewater sulfate – indicates that chronically elevated sulfate deposition had increased the methylation efficiency of the SRB community. The cumulative effect of elevated sulfate deposition to peatlands is to create more effective conditions for methylation and stronger sources of MeHg within a landscape. Furthermore, the fact that changes in sulfate, MeHg, and %MeHg in recovery-treatment porewaters were always intermediate between those in the control and experimental treatments, demonstrates that the effect of elevated sulfate deposition on peatlands persists for some period of time after sulfate deposition has declined.

Table 3.1 Annual precipitation, outflow, and water table elevation (WTE) in the S6 peatland for the periods 1964-2008, 2000-2008, and 2005-2007. Δ WTE is the difference between the annual maxima and minima WTE in the peatland.

	Precip (cm)	Outflow (m^3)	WTE Mean (m a.s.l.)	WTE Min (m a.s.l.)	WTE Max (m a.s.l.)	Median Δ WTE (cm)	Max Δ WTE (cm)
1964-2008	77.30	13832	422.675	422.200	423.117	36	66
2000-2008	71.00	9766	422.696	422.282	423.117	40	62
2005-2007	65.95	8128	422.634	422.282	422.729	55	62

Table 3.2 Regression statistics for the sulfate concentrations in the control, recovery, and experimental treatments against the maximum change in WTE (ΔWTE) over the preceding 10-, 20-, 30-, 60-, and 90-day periods and the duration of that change (Δt).

Treatment	Preceding period	r^2	p-value
Control	10-day	0.09	0.01
	20-day	0.19	<0.01
	30-day	0.06	0.07
	60-day	0.28	<0.01
	90-day	0.16	<0.01
Recovery	10-day	0.10	0.14
	20-day	0.17	0.02
	30-day	0.22	<0.01
	60-day	0.32	<0.01
	90-day	0.14	0.05
Experimental	10-day	0.20	0.02
	20-day	0.20	0.02
	30-day	0.15	0.07
	60-day	0.27	<0.01
	90-day	0.40	<0.01

Multiple regression equation: $\log [SO_4^{2-}] = \max \Delta WTE * \max \Delta t + b + \varepsilon$

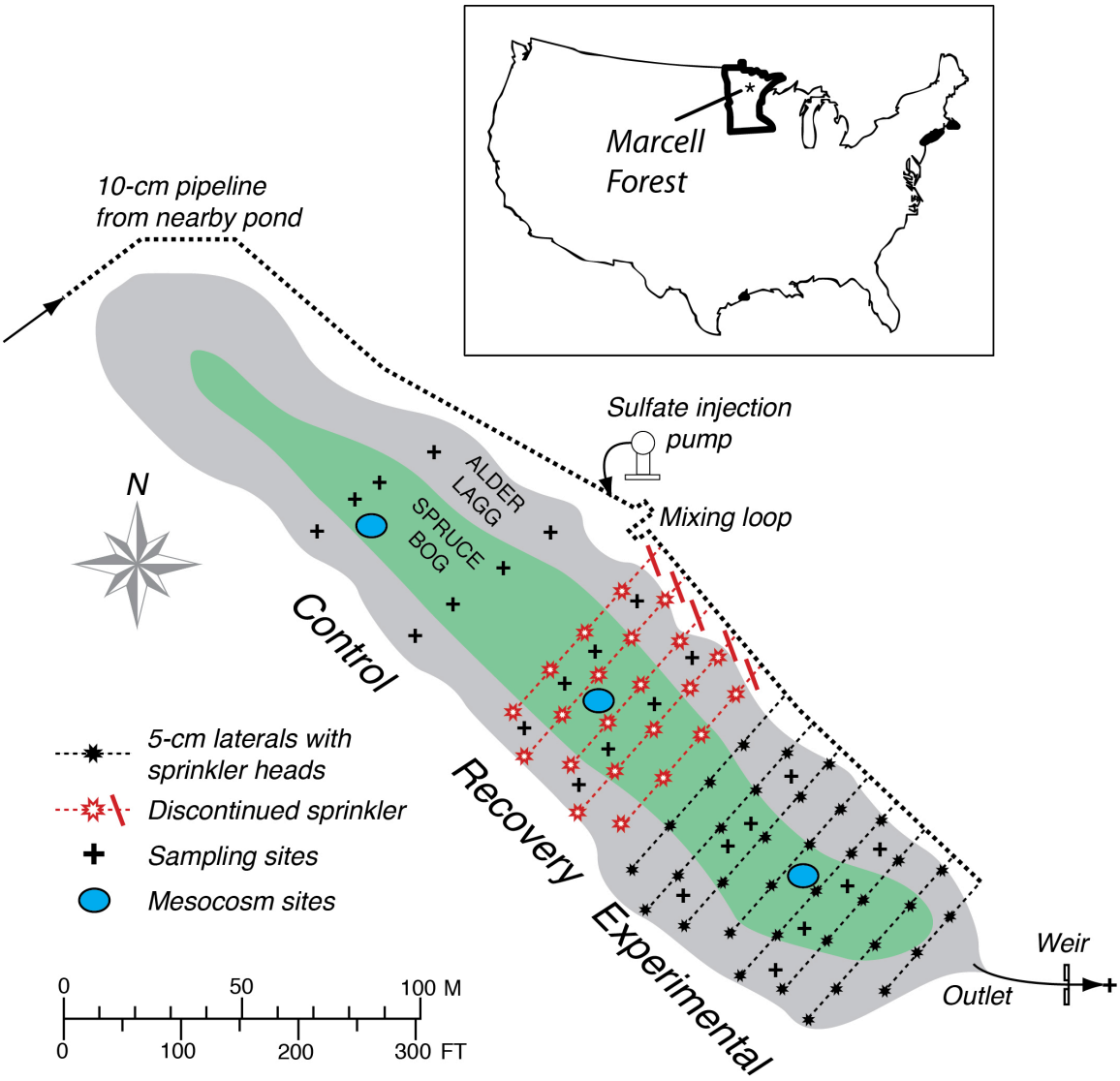

Figure 3.1 A schematic of the experimental design within the S6 peatland illustrating the PVC rainfall simulator, location of sampling sites, and experimental mesocosm locations. See text for details. The inset map shows the location of the Marcell Experimental Forest Minnesota.

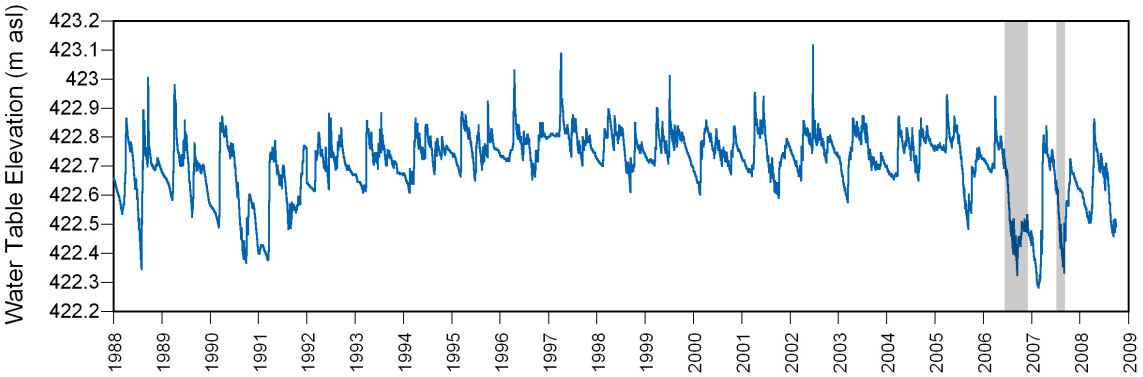

Figure 3.2 Record of water table elevation in the S6 peatland (1988-2008). Shaded bands denote the severe droughts that occurred during the course of the sulfate addition experiment.

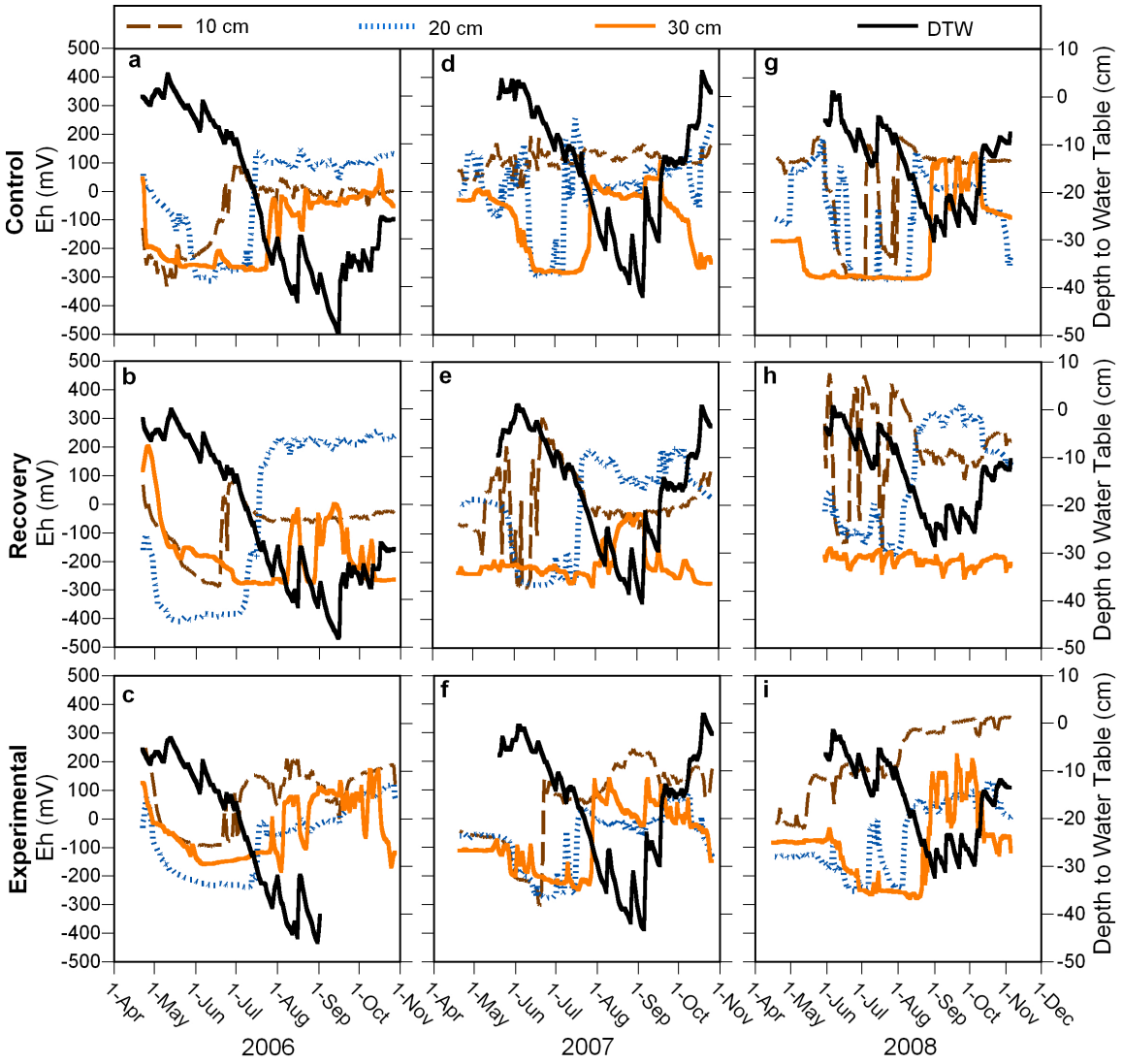

Figure 3.3 Eh profiles at 10-, 20-, and 30-cm depths and depth to water (DTW) from the peat surface in the control, recovery, and experimental treatments in 2006, 2007, and 2008.

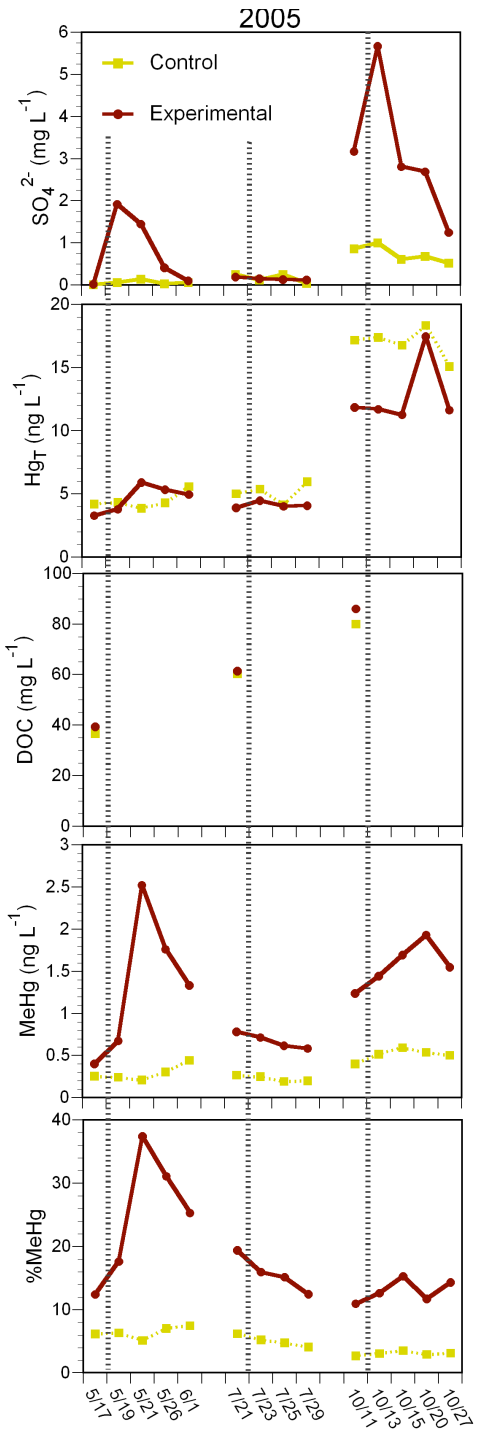

Figure 3.4 Porewater chemisty in the S6 peatland in 2005 (May-October). Dashed lines indicate experimental sulfate additions.

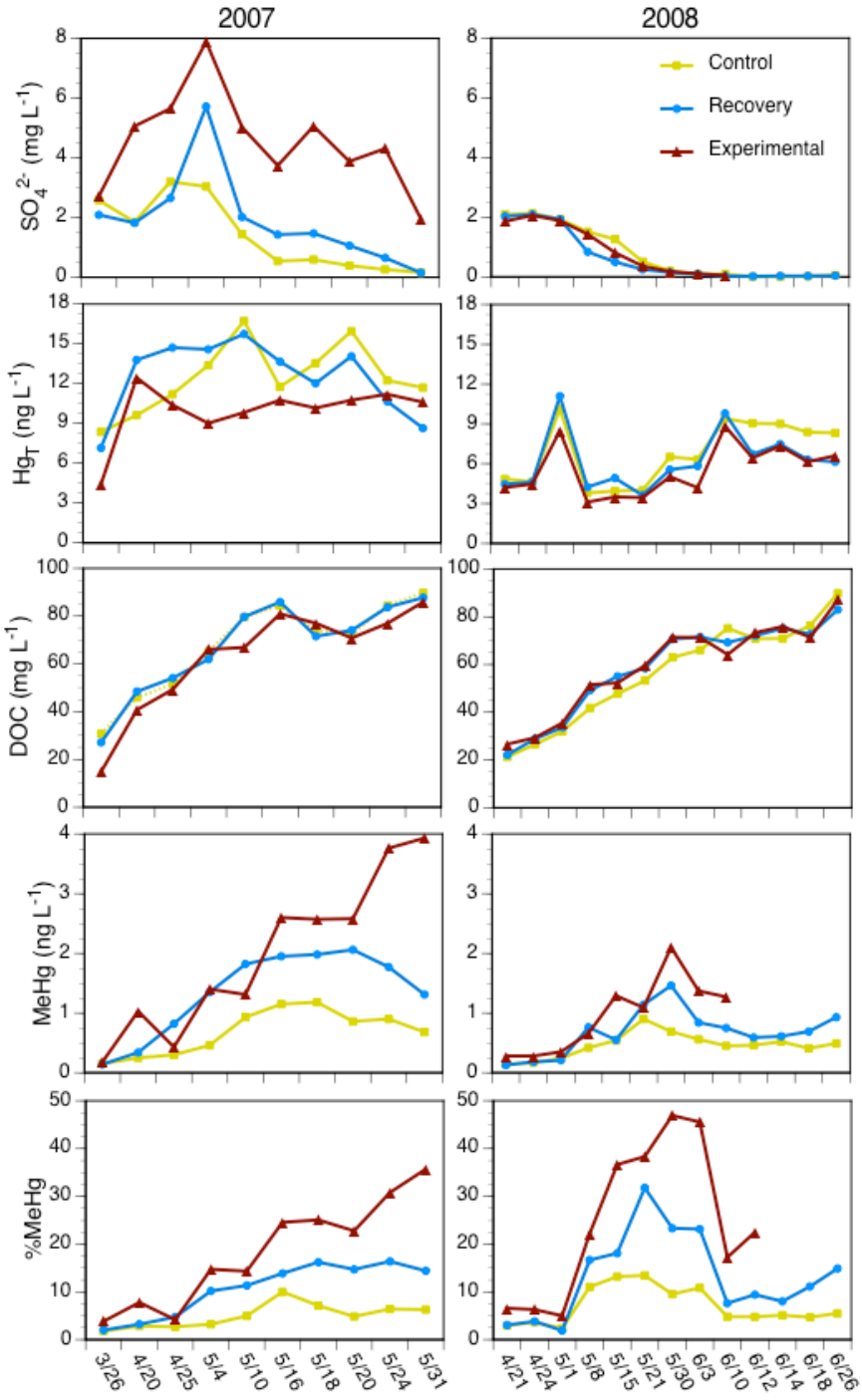

Figure 3.5 Porewater chemistries in each treatment of the S6 peatland over the spring-thaw and sulfate addition periods in 2007 and 2008. Only pre-addition data are shown for sulfate, MeHg, and %MeHg levels in the experimental treatment.

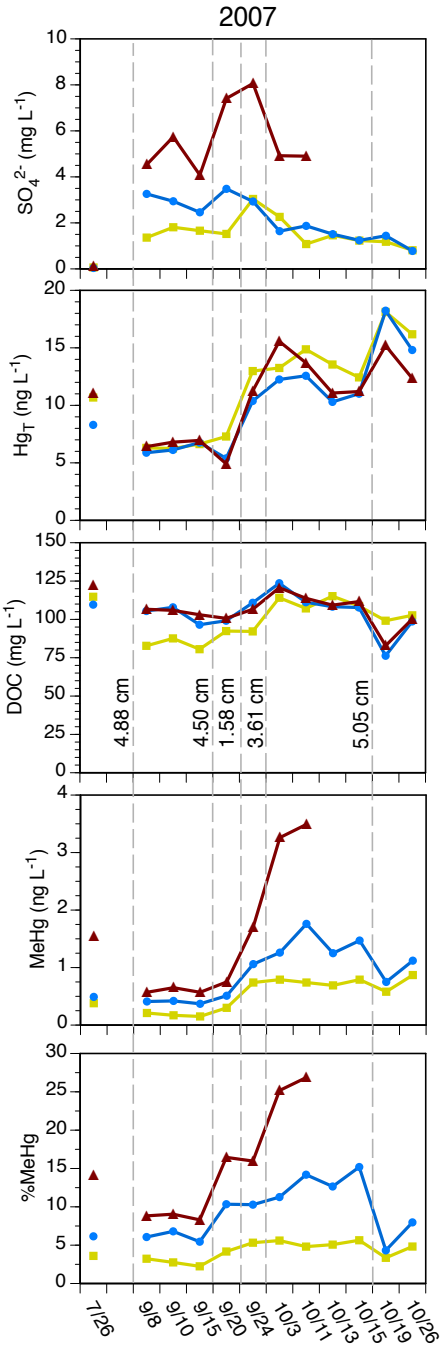

Figure 3.6 Porewater chemistries in each treatment of the S6 peatland over the fall water table rise in 2007. Only pre-addition data are shown for sulfate, MeHg concentrations and %MeHg levels in the experimental treatment. Major rainfall events are indicated by dashed lines and depths (cm).

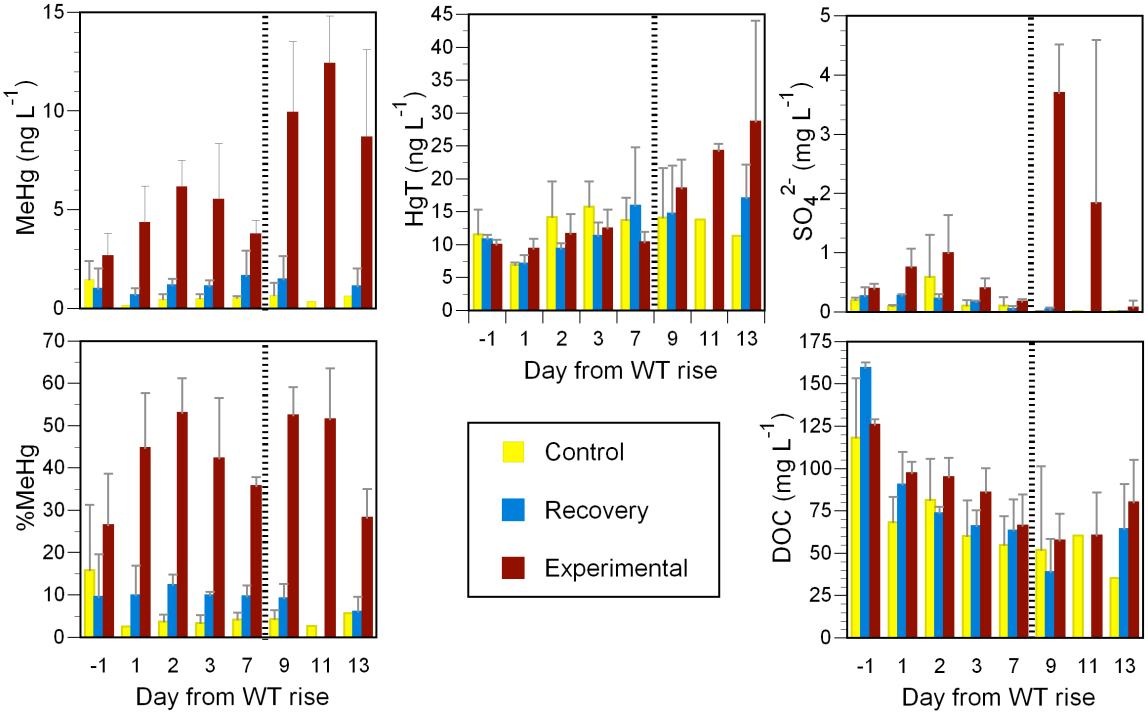

Figure 3.7 Porewater chemistries in the water-table mesocosms in each treatment. Dashed lines indicate experimental sulfate additions to the experimental treatment.

Chapter 4

Methylmercury declines in a boreal peatland when experimental sulfate deposition decreases

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4.1 Summary

Between 2001 and 2008 we experimentally manipulated atmospheric sulfate-loading to a small boreal peatland and monitored the resulting short and long-term changes in methylmercury (MeHg) production. MeHg concentrations and %MeHg (fraction of total-Hg (Hg_T) present as MeHg) in the porewaters of the experimental treatment reached peak values within a week of sulfate addition and then declined as the added sulfate disappeared. MeHg increased cumulatively over time in the solid-phase peat, which acted as a sink for newly produced MeHg. In 2006 a "recovery" treatment was created by discontinuing sulfate addition to a portion of the experimentally-treated section to assess how MeHg production might respond to decreased sulfate loads. Four

years after sulfate additions ceased, MeHg concentrations and %MeHg had declined significantly from 2006 values in porewaters and peat, but remained elevated relative to control levels. Mosquito larvae collected from each treatment at the end of the experiment exhibited Hg_T concentrations that reflect MeHg levels in the peat and porewaters where they were collected. The proportional responses of invertebrate Hg_T to sulfate deposition rates demonstrates that further controls on sulfur emissions may represent as an additional means of mitigating Hg contamination in fish and wildlife across low-sulfur landscapes.

4.2 Introduction

Atmospheric sulfate deposition increased dramatically with the advent of the industrial period, ultimately causing widespread ecosystem acidification, especially downwind of large population centers in North America and Europe (Likens and Bormann, 1974; Rodhe, 1989). Regulatory efforts aimed at controlling sulfur dioxide emissions were very successful at reducing sulfate deposition (Driscoll et al., 2001; Mitchell et al., 2011; Schopp et al., 2003), but ecosystems have responded variably depending on landscape and climatic factors (Stoddard et al., 1999), While most research in sulfate-impacted systems has focused on recovery from environmental acidification (Dillon et al., 2003; Keller et al., 2003), sulfate deposition is also of considerable consequence to the production of methylmercury (MeHg) (Jeremiason et al., 2006), the predominant form of mercury that bioaccumulates in food webs.

Wetlands are a major linchpin in the coupled biogeochemical cycles of sulfur and mercury and serve two potential countervailing roles in ecosystem recovery from sulfate deposition. They are sites of active sulfate reduction and so provide an important sink for legacy sulfate leaching from upland soils toward downstream aquatic systems (Urban et al., 1989). Wetlands are also important sites of mercury methylation in the landscape (St. Louis et al., 1994). Augmented sulfate inputs can stimulate MeHg production in sulfur-limited systems due to the increased activity of sulfate-reducing bacteria (SRB), which are known mediators of the methylation process (Benoit et al., 1999; Branfireun et al., 2001; Branfireun et al., 1999; Gilmour et al., 1992; Gilmour et al., 1998; Jeremiason et al., 2006). Therefore continued inputs of sulfate from uplands may prolong elevated MeHg production in, and export from, wetland systems (Mitchell et al., 2008b). Our understanding of how MeHg production in ecosystems responds to declining sulfate deposition, and the subsequent effects on mercury concentrations in biota, is limited to a handful of largely correlative studies in lakes (Drevnick et al., 2007; Hrabik and Watras, 2002). We therefore lack an experimental basis for predicting the rate of ecosystem recovery, the factors that enhance or inhibit it, or the biogeochemical mechanisms involved.

To investigate the *in situ* response of net MeHg production as an ecosystem recovers from elevated sulfate deposition, we experimentally amended a peatland in northern Minnesota with sulfate for four years and then monitored the system over an equivalent period after sulfate additions ceased. Changes in porewater, peat, and biotic MeHg levels across treatments with differing sulfate depositional histories were used to

1) understand the impacts of increasing and decreasing sulfate deposition on net MeHg production within the peatland, 2) identify mechanisms that promote and inhibit recovery of systems previously impacted by elevated levels of sulfate deposition, and 3) connect changes in sulfate deposition to mercury levels in biota. The extended nature of this project provided an opportunity to study wetland recovery processes against a backdrop of variable climate and hydrology.

4.3 Methods

4.3.1 Study site

This study was performed in the S6 watershed of the Marcell Experimental Forest (MEF), a field-research facility of the Northern Research Station of the USDA Forest Service (Figure 4.1). The 2.0-ha S6 peatland has an overstory of mature black spruce (*Picea mariana*) and tamarack (*Larex laricina*) within a central bog area and is dominated by alder (*Alnus rugosa*) within its lagg margin.(Kolka et al., 2011) The perched water table in the central bog is hydrologically isolated from the uplands and the lagg, creating a mineral-poor, ombrotrophic system ideal for experimental manipulation of atmospheric deposition.

4.3.2 Sulfate addition experiment

Long-term atmospheric deposition records from the National Atmospheric Deposition Program (NADP) site (MN-16) at MEF show that sulfate deposition decreased by roughly 50%, from 11 kg ha^{-1} yr^{-1} in the early 1980s to approximately 5.5 kg ha^{-1} yr^{-1} in the mid-2000s (Appendix B, Figure B1) (NADP, 2011). Our experimental

additions increased sulfate loading to 32 kg ha^{-1} yr^{-1}, or approximately 4x the average ambient, 1990s deposition rate at MEF. This rate is representative of late 20th-century sulfate deposition across large areas of eastern North America, and thus provides an appropriate model for the effects of increasing sulfate deposition on MeHg production as well as the recovery processes that a sulfate-impacted peatland would experience as sulfate deposition declined.

The specific details of the initial experimental design and sulfate delivery system for this study were described previously by Jeremiason et al. (2006) Briefly, in the summer of 2001 the peatland was divided into control and experimental sections, and a sulfate delivery system was constructed of PVC pipe across the down-gradient experimental half (Figure 4.1). Source water was pumped from a nearby, dilute pond (specific conductivity = 20 μS cm^{-1}), a concentrated sodium sulfate solution was injected into the 10-cm main pipeline just above the experimental treatment, and the sulfate-enriched solution was sprayed onto the peatland surface via sprinkler heads atop 1-m risers. Sulfate amendments began in the fall of 2001 and continued three times each year (spring, summer, and fall) through 2008. Each sulfate addition simulated approximately 6-8 mm of rainfall, which did not significantly alter the peatland water table. In the early spring of 2006 a recovery treatment was created by discontinuing sulfate addition to the up-gradient, one-third of the original experimental treatment (Figure 4.1).

4.3.3 Field sampling

4.3.3.1 Porewaters

Two porewater sampling transects were established in the control and experimental treatments, with four 1-m^2 sample plots distributed evenly across the central bog area and lagg margins along each transect (Figure 4.1). To isolate the effect of atmospheric sulfate deposition on MeHg production from effects caused by upland inputs, only data from the central bog sites were considered for this paper. In 2006 two additional transects were established in the newly created recovery treatment, and transects located in the experimental treatment were repositioned down-gradient to ensure sampling occurred well within the treated area. Peat porewater samples were collected from each plot on day -1, +1, +3, and +7 relative to each sulfate addition. Extra sampling days were added to spring and fall samplings on days -7 and +14.

Porewater samples were collected by portable peristaltic pump through a 1.9-cm ID, Teflon probe with a custom-machined tip perforated with 5-mm holes. The probe was inserted into the peat to a depth approximately 5 cm below the water table and porewater was pumped via Teflon tubing through acid-washed, 47-mm Teflon filter-holders (Savillex Co.) fitted with ashed, 0.7-µm, glass-fiber filters directly into new, 125-mL PETG bottles. Bottles were rinsed in triplicate with porewater prior to filling, and samples were preserved with high purity HCl to 0.5% (vol/vol). Samples were collected for dissolved Hg_T, MeHg, and major anions on each sampling day throughout the course of the project. Hg_T and MeHg samples were collected using accepted clean sampling techniques (Bloom and Fitzgerald, 1988). Field duplicates and equipment blanks accounted for 10% of samples.

4.3.3.2 Peat samples

Surficial peat cores were collected annually from each treatment in 2003, 2005-2007, and 2009 by coring or cutting and hand-collection (Appendix B, Table B1). All peat samples were kept in frozen storage and freeze-dried prior to analysis of Hg_T and MeHg.

4.3.3.3 *Invertebrate Samples*

In late spring 2009, near the end of the study, mosquito (*Culex* spp.) larvae were collected in triplicate batches from each treatment by netting with vinyl-coated aquarium nets. Mosquito larvae were hand-picked at the MEF laboratory, placed in vials of deionized water overnight to purge gut contents, and then frozen. Samples were freeze-dried prior to analysis of Hg_T content. Where enough mass remained, samples were also analyzed for MeHg content.

4.3.4 Laboratory analyses

4.3.4.1 Dissolved mercury

Aqueous Hg_T was analyzed according to EPA method 1631 Revision E. (US-EPA, 2002) Samples were oxidized overnight with BrCl and then neutralized with NH2OH. Stannous chloride reduced the oxidized mercury species to Hg^0, which was purged and trapped on gold traps. Mercury was thermally desorbed from the traps in a stream of Ar and analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS) on a Tekran 2600 Automated Total Mercury Analyzer. Daily calibrations were checked with

lab-made standards. Each run included 20% deionized-water blanks, 10% sample duplicates, and 5% sample matrix spikes.

Aqueous MeHg was analyzed according methods described in Bloom(1989) and Liang et al.(1994) at the Branfireun laboratory (2005 samples), the Jeremiason laboratory (2006 samples), or the Balogh laboratory (2007 and 2008 samples). Samples were distilled with 8M H2SO4 and 20% KCl in an acid-cleaned, Teflon, extraction manifold and distillates were analyzed within 48 hours. Mercury species were ethylated with sodium tetraethylborate and then purged from solution and trapped on Tenax traps. Mercury species were thermally desorbed from the traps and carried in a stream of Ar or He through a short chromatographic column. The separated mercury species passed through a pyrolytic trap where they were thermally transformed into Hg^0, and analyzed by CVAFS on a Tekran 2500 spectrometer (Branfireun and Jeremiason laboratories) or a Brooks Rand Model III (Balogh laboratory). Each run included 5% deionized-water blanks, 10% sample duplicates, and 5% sample matrix spikes.

4.3.4.2 Dissolved anions

Water samples for major anions (SO_4^{2-}, Cl^-, Br^-) were analyzed on a Dionex DX-500 ion chromatograph according to standard methods by the USFS Northern Research Station laboratory in Grand Rapids, Minnesota. Each run included 10% deionized-water blanks, 10% sample duplicates, and check standards. Replicate standard measures and lab duplicates were within 10% and method detection limits were 0.1 mg L^{-1} each year.

4.3.4.3 Solid phase mercury

For Hg_T analysis, peat samples were microwave digested in concentrated HNO_3 and diluted prior to analysis by dual gold-trap amalgamation CVAFS, as described above for porewaters. For MeHg analysis, peat samples were distilled as outlined for porewaters, but with the inclusion of a known mass spike of enriched $Me^{199}Hg$ in each vessel. Samples were analyzed by isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry (ID-GC-ICPMS) with detection mercury on an Agilent 7700 ICPMS according to the methods of Hintelmann et al.(1995) In addition to blanks and duplicates, certified reference materials (MESS-3 for Hg_T; ERM-CC580 for MeHg) were analyzed in 10% of samples.

Quality assurance and control results for aqueous and solid phase Hg_T and MeHg for each year can be found in Tables B2-B4 of Appendix B.

4.3.4.1 Biological mercury

For Hg_T analysis, mosquito larvae samples were microwave digested in concentrated HNO3 and diluted prior to analysis by dual gold-trap amalgamation CVAFS, as described for porewaters. MeHg in mosquito larvae samples was heat extracted in a solution of 25% KOH in methanol, with a known mass spike of enriched $Me^{199}Hg$ in each vessel. Samples were analyzed by ID-GC-ICPMS. In addition to blanks and duplicates, the certified reference material DORM-3 was analyzed in 10% of samples.

4.3.5 Numerical analyses

Weighted means were calculated for annual porewater results because sampling dates were not evenly distributed throughout the season. Annual porewater values from each treatment were calculated by multiplying the mean result on each sampling day within a treatment by a weighting factor and then summing. The weighting factor was equal to the fraction of the season represented by a sample since the previous sampling date (e.g. the day -1 sample collected for a summer addition had a much larger weighting factor than a sample collected 2 days later on day +1). The season began on the first date on which peat soil temperatures at 10-cm depth were greater than 1ºC, and ended with the last sampling date each year. Bulk density of the peat did not change appreciably within the top 8 cm (one-way Anova, $p=0.18$), and so mean results for each peat core were calculated by multiplying concentrations for each interval by a weighting factor related to interval thickness (2 or 4 cm) and summing. Treatment means were then calculated from the weighted averages. Mosquito larvae results from each sample batch were averaged for each treatment.

The program R was used for all statistical analyses (R-Development-Core-Team, 2011). The distributions for both porewater and solid data were right-skewed, so each data set was natural-log-transformed prior to statistical analyses to obtain a normal distribution. A linear-least-squares model of the transformed data was fit on treatment and year factors. Residual plots of the transformed data did not show any systematic bias. General linearized hypothesis tests were used to compare the estimated slopes for each

treatment in each year and generate p-values. A p-value <0.05 was considered significant.

4.4 Results

4.4.1 Effect of sulfate addition

4.4.1.1 Porewaters

An increase in porewater MeHg concentration in response to sulfate addition was clearly evident following spring sulfate application to the central-bog as illustrated here for the spring of 2006 and 2008 (Figure 4.2), the first and last year of recovery respectively. In each year porewater sulfate concentrations in the experimental treatment peaked one day following the additions (2.9 ± 2.1 mg L^{-1} in 2006 and 3.8 ± 2.2 mg L^{-1} in 2008). As sulfate concentrations declined, the porewater MeHg pool increased dramatically (Figure 4.2a). MeHg concentrations peaked by the third day post-addition in each year (4.3 ± 2.1 ng L^{-1} in 2006 and 3.6 ± 1.0 ng L^{-1} in 2008). MeHg as percentage of Hg_T (%MeHg) followed a very similar pattern, peaking at 46 ± 29% three days after the addition in 2006 and at 50 ± 22% seven days after the addition in 2008 (Figure 4.2b). In contrast, mean sulfate and MeHg concentrations and %MeHg in the control area were consistently low each spring (< 0.5 mg L^{-1}, < 0.6 ng L^{-1}, and < 7%, respectively). MeHg concentrations and %MeHg were significantly higher in the experimental treatment than in the control on each day shown in Figure 4.2 ($p < 0.05$). Peak MeHg concentrations and %MeHg in the experimental treatment, post-addition, were significantly higher than pre-addition levels ($p < 0.05$). Annual, seasonally-weighted average MeHg

concentrations and %MeHg in the experimental treatment were 4-9x higher than corresponding levels in the control section (Figure 4.3).

4.4.1.2 Peat

The solid phase data integrate the responses to sulfate additions that were noted above for porewater MeHg concentrations and %MeHg in the experimental treatment (Figure 4.2). In the control section, MeHg concentrations and %MeHg remained consistently low in both peat and porewaters (Figure 4.3). Average MeHg concentrations and %MeHg in the peat of the experimental treatment were 4-9x greater than the corresponding values in the control section. There was no significant effect of treatment on Hg_T concentrations in peat, which ranged between 63 and 110 ng g^{-1} across the peatland over the 5-year period.

4.4.2 Recovery treatment trends

4.4.2.1 Porewaters

The recovery treatment – a sub-section of the experimental treatment to which sulfate application was halted – was created in the spring of 2006. Sulfate concentrations in recovery porewaters declined almost immediately thereafter, generally remaining low and following a temporal pattern similar to that of the control in each year (Figure 4.2a). In contrast to sulfate, MeHg concentrations and %MeHg in recovery treatment porewaters remained elevated well above control levels during the first year of recovery ($p< 0.001$). In 2007 annual, seasonally-weighted %MeHg declined 37% from 2006 levels ($p<0.001$), but then held steady between 2007 and 2009. MeHg concentrations fell more

gradually over the recovery period, declining 32% between 2006 and 2008 ($p<0.001$). Both MeHg concentrations and % MeHg in the recovery section remained elevated relative to control values through the end of the study (Figure 4.3).

4.4.2.2 Peat

MeHg concentrations and %MeHg in recovery treatment peat declined by 62% and 76%, respectively, between 2006 and 2009 ($p < 0.005$ and $p < 0.02$). The solid peat represented the major sink for MeHg and Hg_T – of the total mercury mass in the upper 8 cm of peat matrix, >99.7% of MeHg and >99.8% of Hg_T was bound to the peat.

4.4.3 Biotic mercury

Dry-weight, Hg_T concentrations in *Culex* spp. larvae mimicked %MeHg trends in peat samples, with experimental-treatment larvae having significantly elevated mercury concentrations relative to those found in the control and recovery sections ($p < 0.05$; Figure 4.5). Significant differences in mosquito-larvae Hg_T also persisted between the control and recovery sections ($p < 0.05$). Although sample masses were insufficient to allow MeHg analysis of all mosquito larvae samples, for the six samples measured for both Hg_T and MeHg in this study, MeHg comprised 62 ± 19% of Hg_T in mosquito larvae, and Hg_T explained 75% of the variability in MeHg concentrations (Appendix B, Figure B2).

4.5 Discussion

4.5.1 MeHg response to sulfate applications

The short and long-term processes whereby elevated sulfate deposition affected MeHg production within the S6 peatland were explored through intensive sampling of porewaters and periodic collections of peat cores, respectively (Figure 4.1). While the MeHg pool in porewaters can be affected by factors other than methylation, such as changes in water chemistry, partitioning between the aqueous and solid phases, and the character and abundance of organic ligands (Gilmour et al., 1998; Miller et al., 2007; Skyllberg, 2008), MeHg in porewater nevertheless represents the most dynamic and mobile MeHg pool and is thus important for considering downstream effects.

4.5.1.1 Porewaters

The order-of-magnitude increases in MeHg concentrations and %MeHg in porewaters of the experimental treatment following sulfate application are of similar magnitude and timing to the responses reported by Jeremiason et al. (2006) for the first year of this study and other mesocosm-scale studies in nutrient-poor, boreal peatlands (Branfireun et al., 1999; Mitchell et al., 2008a). Our interpretation of these results is that the added sulfate stimulated SRB activity resulting in a net increase in Hg methylation. The steady buildup of a large pool of solid-phase MeHg in the peat matrix presented above provides strong evidence for this de novo production of MeHg.

An alternative explanation for the observed increase in porewater MeHg is a change in partitioning of MeHg and Hg_T between the aqueous and solid phase resulting

from an increase in the dissolved sulfide pool (Skyllberg, 2008). Mercury speciation was modeled in response to increasing dissolved sulfide concentrations and found that the molar ratio of MeHg to Hg_T peaked at 0.3 μM sulfide and subsequently decreased, which is similar to previously reported findings (model parameters shown in Appendix B, Table B5) (Skyllberg, 2008). However, at low sulfide concentrations the model did not accurately predict MeHg and Hg_T concentrations in the dissolved phase possibly because of uncertainty in the log K value for the reaction between MeHg and thiol groups or because of kinetic limitations controlling adsorption/desorption of MeHg. Many studies have demonstrated the difficulty of accurately representing mercury speciation in the presence of high DOC (Drexel et al., 2002; Hsu and Sedlak, 2003; Miller et al., 2007; Ravichandran, 2004). Although we can not rule out the possibility that sulfide-driven changes in solid-phase partitioning caused porewater MeHg to increase, the weakness of the simple equlibrium model and the fact that the total pool of MeHg in the experimental section increased progressively over time argues strongly that increased MeHg production, rather than sorption/desorption reactions, is responsible for the MeHg patterns seen following sulfate addition.

4.5.1.2 Peat

The MeHg pool within a peatland represents a dynamic equilibrium between MeHg production, predominantly through biotic methylation, and removal processes, including biotic and abiotic demethylation, bioaccumulation, and advective transport (Branfireun et al., 1999; Gilmour and Henry, 1991; Gilmour et al., 1998). In sulfur-limited systems, such as the experimental peatland in this study, sulfate addition

represents an important factor influencing MeHg production and contributes to higher MeHg concentrations in wetland porewaters and soils than would be expected based on atmospheric Hg inputs alone (Benoit et al., 2002; Branfireun et al., 1999; Gilmour et al., 1992; Gilmour et al., 1998). The increases in %MeHg in peat and porewaters of the experimental treatment relative to those in the control indicate that experimentally increasing sulfate loads shifts that equilibrium toward greater MeHg production.

4.5.2 Recovery from elevated sulfate deposition

Demethylation was a more important MeHg loss process than desorption coupled with advective transport out of the system. This conclusion follows from the observation that concentrations of MeHg in porewaters were too low to account for the mass of MeHg lost from the recovery-section peat. Jeremiason et al. (2006) found that nearly 1800 μg MeHg was exported from the S6 peatland in 2002. The mass of MeHg lost in the top 8 cm of the recovery treatment alone between 2006 and 2009 was approximately 120 mg, or more than 65x the amount exported in outflow in 2002 from the entire peatland.

Methylmercury concentrations in the peat of the recovery treatment did not show significant declines within the first two years after sulfate additions were halted. This could either imply that the kinetics of desorption of the newly accumulated MeHg from the peat was much slower than the decreases in methylation rates in porewaters (thereby maintaining the chronic observed difference in porewater MeHg between the control and recovery treatments) or that elevated MeHg production was sustained for a period of time by internal recycling of the previously added sulfate. Such recycling has been proposed by others (Branfireun et al., 1999; Gilmour et al., 1998) and would also explain our

observed short-term response to sulfate addition in which sulfate disappeared from experimental porewaters within three days of application, while porewater MeHg levels remained elevated two weeks later (Figure 4.2). Urban et al.(1989) investigated sulfur biogeochemistry in a small peatland 1 km from the S6 site and determined that annual recycling of sulfur was equivalent to annual external sulfur inputs. Blodau et al.(2007) found evidence that an anaerobic sulfur cycle sustained SRB activity under reducing conditions in an ombrotrophic peatland, providing an explanation for the high sulfur recycling rates observed by Urban et al. (1989) Thus one possible mechanism for recovery following the cessation of sulfate addition to the S6 peatland is that sulfur compounds within the peat become more recalcitrant over time. That is, as the pool of added sulfur is repeatedly turned over, labile sulfur compounds are preferentially consumed and progressively converted into refractory organic forms, which are much more slowly cycled by anaerobic and aerobic processes. In line with this hypothesis, differential sulfate release was observed among treatments in the S6 peatland following drying events (as described in Chapter 3), which can expose reduced sulfur moieties to oxygen. The highest sulfate release into porewaters occurred in the experimental treatment, and the lowest release was observed in the control section. Because there was no significant difference among treatments in size of the total sulfur pool in the peat, these results suggest that the newly added sulfate was more susceptible to release/recycling than the pre-existing pool of ambient sulfur.

4.5.3 Interannual variability

Despite the significant trends in peat MeHg concentrations and %MeHg (increases in the experimental treatment and decreases in the recovery treatment), there is some unexplained variability in the data – for example, the decrease in peat %MeHg between 2003 and 2005 and the fluctuating porewater values in the experimental treatment (Figure 4.3). These variations are likely the result of year-to-year differences in precipitation and hydrology, such as the series of summer droughts that persisted at the MEF from 2005 to 2007. Hydrologic variability can affect mercury cycling in peatlands by altering peat accumulation and decomposition, redox conditions, and methylation potentials.(Balogh et al., 2006; Brigham et al., 2002; Hall et al., 2005; St. Louis et al., 2004) Such effects are most clearly evident in the S6 control treatment where interannual fluctuations in both porewater and peat MeHg cannot be the result of sulfate manipulation. In the experimental and recovery treatments the effects of these large-scale physical processes are superimposed on trends due to sulfate addition alone. For example, the 2007-2009 decline of MeHg in the recovery section can be explained, at least in part, by the cessation of sulfate amendments, but this should not be the case for the experimental treatment where sulfate additions continued. Thus it appears that some of the interannual variability in MeHg concentrations and %MeHg in each treatment (Figure 4.3) was the result of overriding climatic and/or hydrologic effects.

To remove the influence of natural hydrologic variability from the longer-term effects of experimental sulfate addition, we normalized MeHg concentrations and %MeHg in the experimental and recovery treatments to corresponding values in the

control treatment for porewaters and peat in each year (Figure 4.4). Normalized MeHg concentrations and %MeHg in the experimental peat increased cumulatively with time such that by 2009 these values in the experimental treatment were 5-6x higher than those of the control ($p < 0.005$). In the recovery treatment the opposite trend occurred, and by 2009 normalized MeHg concentrations and %MeHg approached a value of 1, indicating a near-return to control levels. However, the trend was not significant ($p = 0.28$) owing to small sample sizes ($n = 4$) from each treatment. Normalized MeHg concentrations in the porewaters of the experimental treatment did not show any discernable trend with time, presumably because most newly produced MeHg accumulated in the peat. The large loss of MeHg from the recovery-section following the discontinuation of sulfate addition indicates that reductions in sulfate deposition could produce a relatively rapid decline in MeHg export to connected lakes and streams.

4.5.4 Biotic response

In the spring of 2009 mosquito larvae (*Culex* spp.) were collected in the S6 peatland to compare mercury concentrations in biota among treatments, as mosquitoes are sensitive indicators of mercury loading to, and MeHg production within, aquatic systems (Hammerschmidt and Fitzgerald, 2005). The biotic results provide direct evidence that increasing/decreasing sulfate loading to peatlands translates into significant increases/declines in biotic mercury concentrations. While MeHg in experimental-treatment peat was >4.5x that in the control by 2009, Hg_T in mosquito larvae from the experimental treatment in the same year was just over 2x the levels found in the control. Apparently some of the MeHg produced as a result of sulfate-stimulation became less

bioavailable with time. This finding agrees with other studies which have found that recently produced MeHg is more available to biota than older MeHg (Harris et al., 2007; Orihel et al., 2008).

Because detritivorous mosquito larvae spend a short time in their aquatic habitat, they present a snapshot of mercury bioaccumulation in the season during which they hatch. Mercury bioaccumulation within sulfate-impacted peatlands may be even greater for invertebrates with long aquatic larval stages and those higher in the food chain, such that recovery from sulfate deposition may take longer than for mosquito larvae. Although the S6 wetland does not itself support fish, its outflow contributes to the MeHg load of downstream lakes that have susceptible fish populations. Moreover, direct transfer of MeHg to terrestrial foodwebs through the emergence and predation of aquatic insects has been identified as an important trophic pathway that may contribute to lowered reproductive success for insectivorous birds that exploit riparian and wetland habitats (Cristol et al., 2008; Custer et al., 2007).

4.6 Conclusions

Our long-term sulfate-loading experiment created an opportunity to observe the *in situ* processes whereby sulfate deposition enhanced MeHg production within a peatland, MeHg declined once sulfate additions were discontinued, and mercury levels in biota mirrored changes in sulfate inputs. Increasing sulfate deposition by 4x led to a MeHg increase of similar magnitude in both porewaters and peat. These changes in MeHg production occurred despite flat trends in Hg deposition over the study period (Risch et

al., 2012). The steady accumulation of MeHg in the peat over time, relative to the control, suggests sustained disequilibrium between methylation and demethylation over the course of the experiment. At what point equilibrium between MeHg production and removal processes would be achieved at these elevated levels of sulfate deposition is an open question. The finding that most of the MeHg lost from the recovery treatment was likely due to *in situ* demethylation rather than export from the system implies that the majority of the MeHg produced in response to elevated sulfate deposition may not be transported to downstream aquatic systems. This is supported by the finding that peat and porewater MeHg increased by ~4x in response to a 4x increase in sulfate deposition but MeHg flux from the wetland in the first year of this study only increased by 2x (Jeremiason et al., 2006).

The proportional, synchronous decreases in mosquito-larvae mercury with cessation of sulfate addition indicate that declines in sulfate deposition can directly reduce MeHg in biota. Wetland recovery from elevated, anthropogenic sulfate deposition may explain some of the downward trends seen in fish and wildlife mercury across North America and Europe in the late 20th century as regulations on sulfur emissions took effect (Chalmers et al., 2011; Drevnick et al., 2007; Evers et al., 2011; Monson et al., 2011). It is important to note that atmospheric mercury deposition declined concurrently with the reductions in sulfate deposition in many areas (Driscoll et al., 2007) and may also be responsible for declining mercury concentrations in biota.

In this study MeHg responses to climatic variability were superimposed on the trends caused by sulfate addition alone. The fluctuations in peat MeHg seen in the control

section, and the declines in MeHg concentrations in the experimental treatment over the periods 2003-2005 and 2007-2009, demonstrate that physical processes can also alter the balance between methylation and demethylation from year to year. Climatic events such as severe droughts, which lead to oxidation of reduced sulfur species and sulfate formation, may slow or reverse declining MeHg levels in wetlands. The influence of drought on sulfate release from wetlands and sulfate export from watersheds are well documented (Bayley et al., 1986; Devito and Hill, 1999; Eimers et al., 2007; Mitchell and Likens, 2011; Warren et al., 2001). Altered sulfur cycling consequent to climatic shifts may thus explain some of the recently reported reversals in downward fish mercury trends noted above (Evers et al., 2011; Monson, 2009).

Sulfate deposition to ecosystems downwind of industrial centers increased by more than an order of magnitude over natural background rates by the mid-20th century (NADP, 2011). It is reasonable to infer that such large increases in sulfate loading caused comparably large increases in MeHg production in sulfur-limited peatlands – increases above and beyond those arising from the 3-4x rise in mercury deposition during that same time period (Lindberg et al., 2007; Munthe et al., 2007). Subsequent regulations of sulfur emissions, such as the 1970 Clean Air Act and its 1990 amendments in the US, led to substantial reductions in sulfate deposition across regions once affected by very high levels of atmospheric loading (Mitchell and Likens, 2011). As of 2009 sulfate deposition across eastern North America remained well above background levels(NADP, 2011) highlighting the potential benefits to additional reductions. Our finding that peatland MeHg responds rapidly to reductions in sulfate inputs implies an opportunity to mitigate

mercury contamination through policies aimed at further reducing sulfur emissions and deposition.

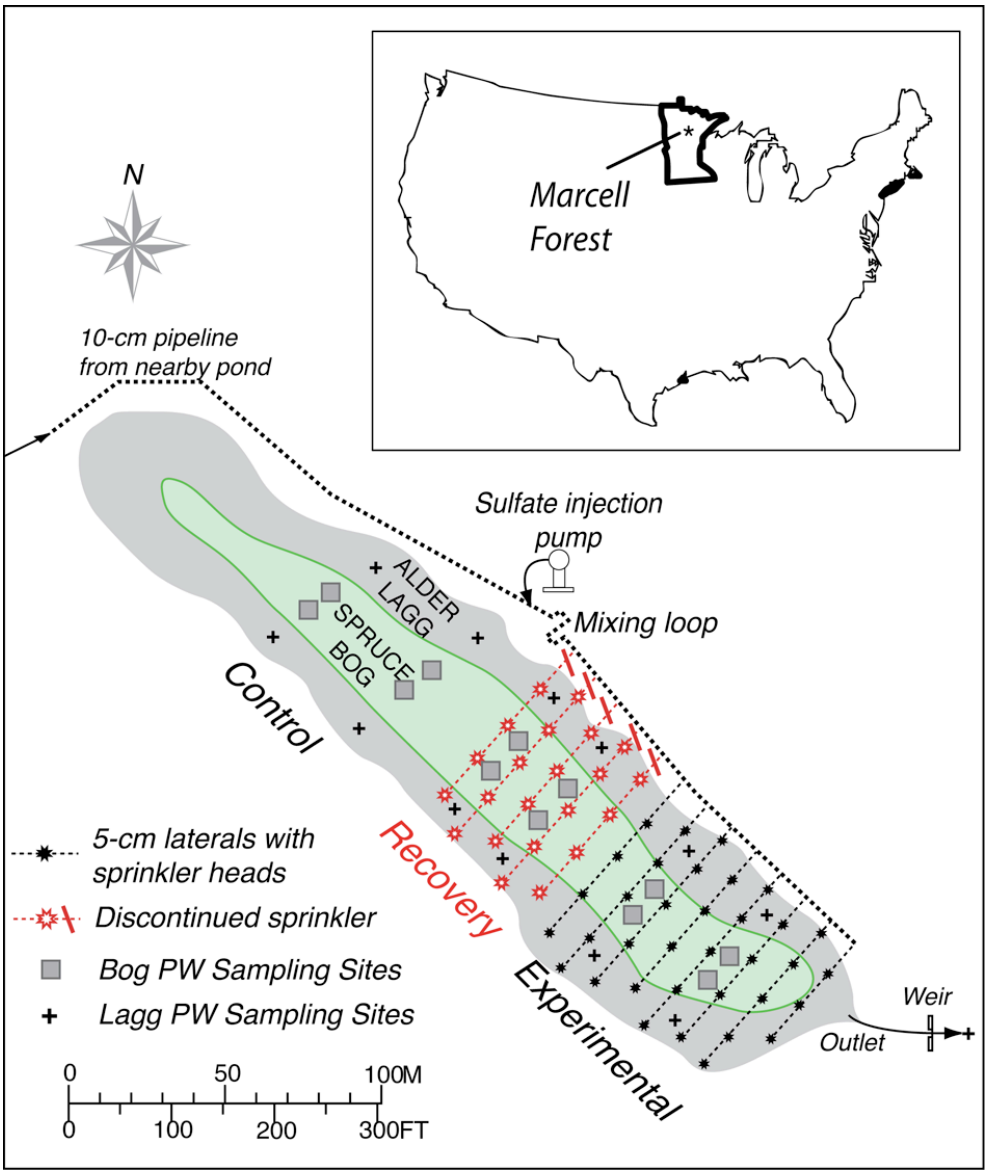

Figure 4.1 A schematic of the sulfate delivery system illustrating the experimental design within the S6 peatland. Porewater (PW) sampling sites in the bog (■) and lagg (+) were located along transects within each treatment. The first 5 lateral pipelines encompass the recovery treatment. See text for further details. The inset map shows the location of the Marcell Experimental Forest.

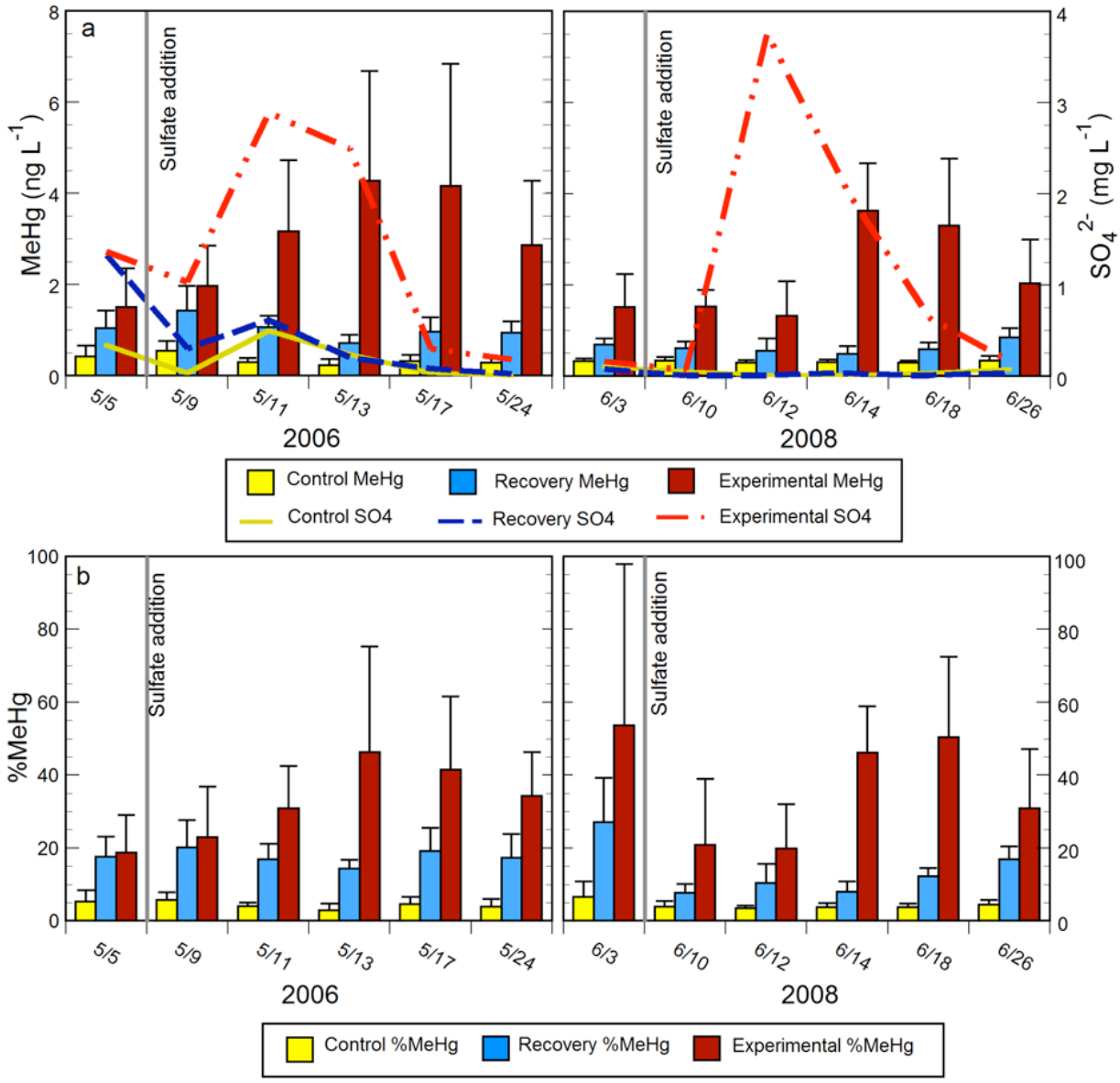

Figure 4.2 a) Sulfate and MeHg concentrations (± 1 s.d.), and b) %MeHg (the ratio of MeHg to Hg_T; ± 1 s.d.) in control, recovery, and experimental treatment porewaters of the S6 peatland over the period of spring sulfate addition in 2006 and 2008. The spring 2006 and 2008 addition periods were chosen because they are the first and last year of recovery respectively.

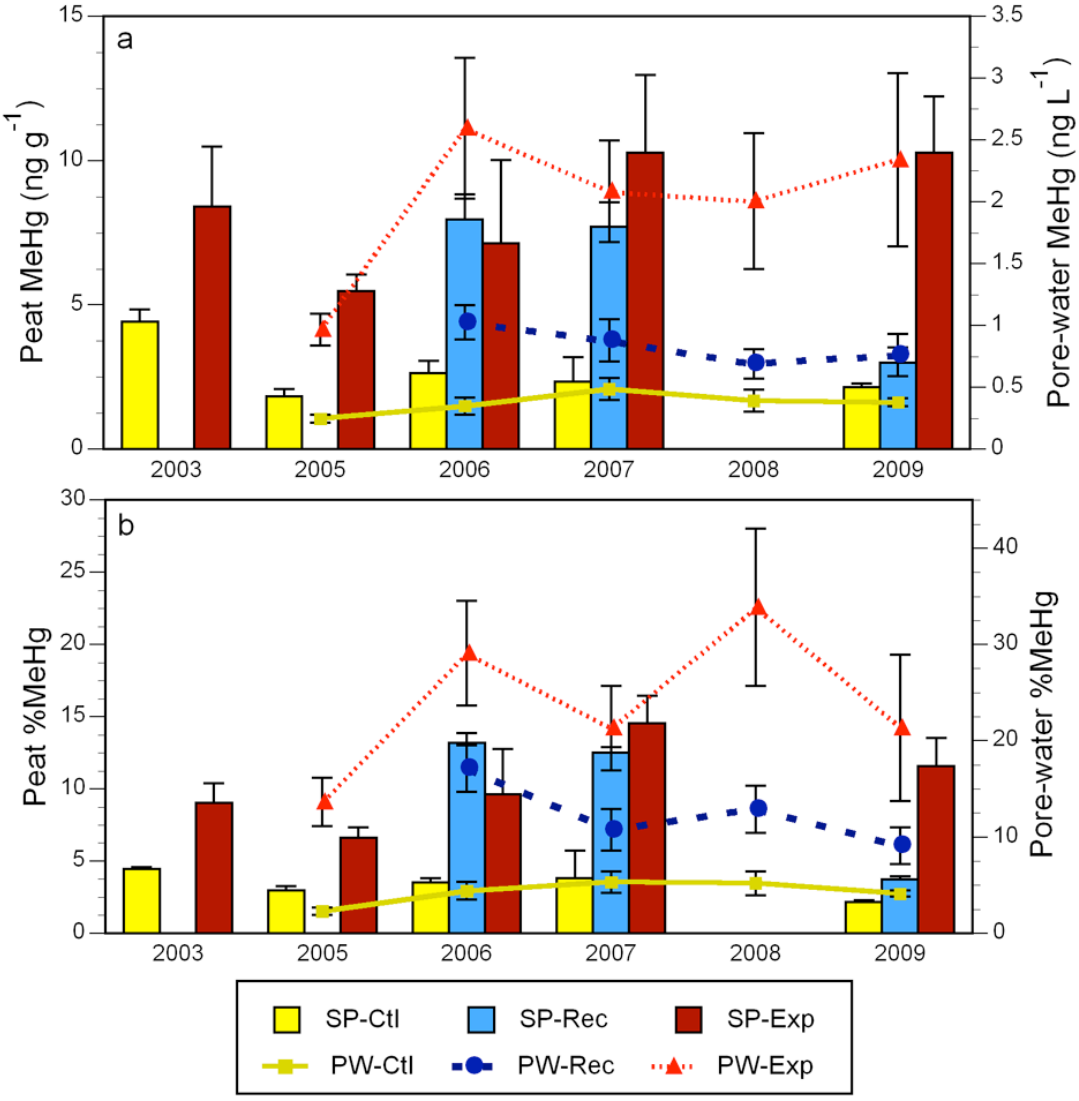

Figure 4.3 a) MeHg concentrations and b) %MeHg levels in the solid peat (SP; interval-weighted average values) and porewaters (PW; annual, seasonally-weighted average values) in the control, recovery, and experimental sections of the S6 peatland 2003-2009. Error bars for peat are standard errors of weighted treatment means. Error bars on porewaters are standard deviations calculated from weighted annual means.

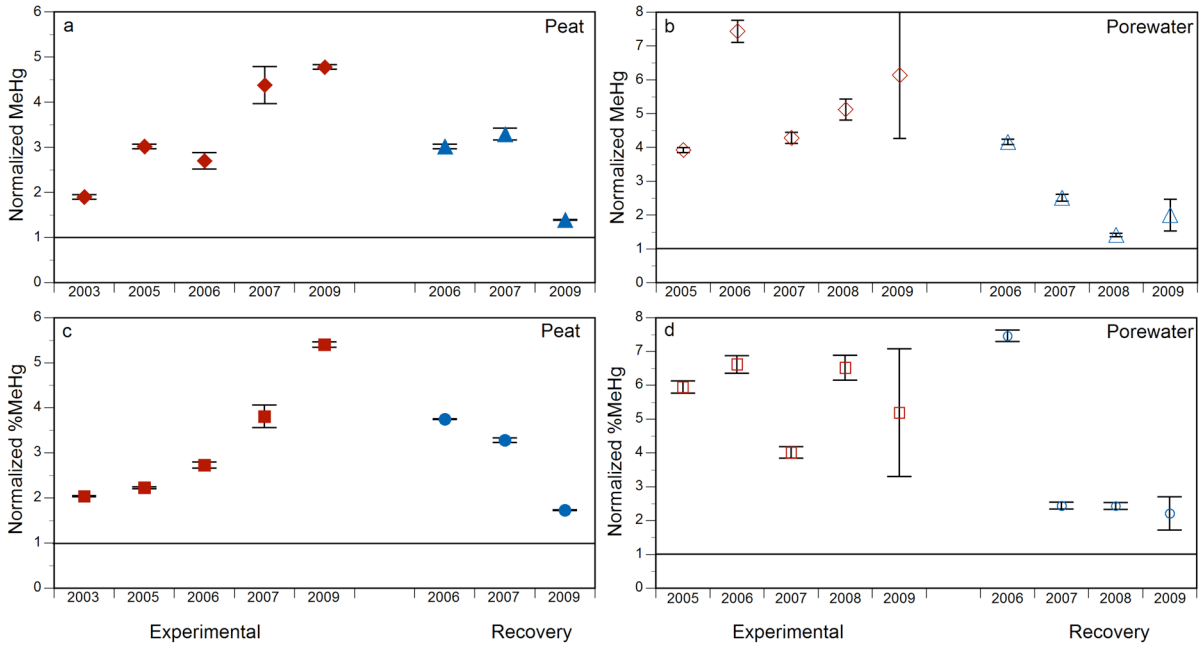

Figure 4.4 Ratio of [MeHg] and %MeHg in recovery and experimental treatments to [MeHg] and %MeHg in the control treatment in the peat (a and c) 2003-2009 and porewaters (b and d) 2005-2009 ([MeHg] experimental peat (♦), [MeHg] experimental porewater (◊), %MeHg experimental peat (■),%MeHg experimental porewater (□), [MeHg] recovery peat (▲), [MeHg] recovery porewater (Δ), %MeHg recovery peat (●), %MeHg recovery porewater (○)). Peat error propagated from standard errors of mean [MeHg] and %MeHg in control and respective treatment (experimental or recovery). Porewater error propagated from standard deviations for control and respective treatment. The horizontal line at y = 1 in each figure represents a ratio of 1:1 or a return to control levels in the treatments.

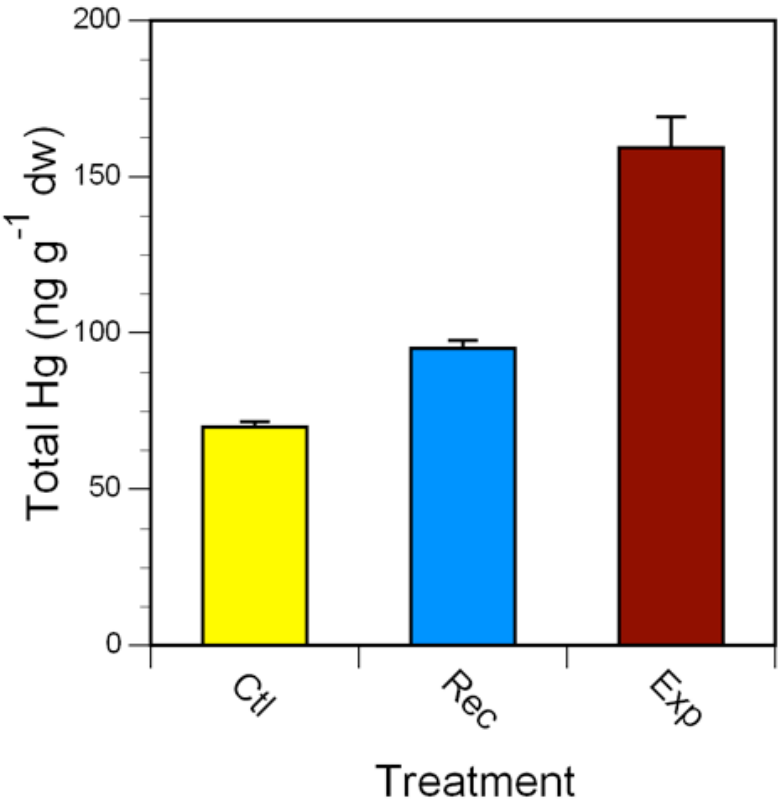

Figure 4.5 Dry-weight, Hg_T concentrations (± 1 s.d.) in mosquito larvae (*Culex* spp.) in control (Ctl), recovery (Rec), and experimental (Exp) treatments in spring 2009.

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Appendix A: Supporting information for Chapter 3

Table A1. QAQC results for Hg_T analyses in porewaters, 2005-2009.

	Percent Spike Recovery	Duplicate Relative Percent Difference	Method Blank ($ng\ L^{-1}$)	Detection Limit ($ng\ L^{-1}$)
2005	98± 6	2 ± 2%	0.22	0.31
2006	93± 14%	3 ± 7%	0.53	0.85
2007	78± 16%	8 ± 5%	0.90	1.10
2008	114± 17%	7 ± 7%	0.79	0.72
2009	92 ± 4	1 ± 1%	N/A	0.22

N/A: samples not analyzed

Table A2. QAQC results for MeHg analyses in porewaters, 2005-2009.

	Percent Spike Recovery	Duplicate Relative Percent Difference	Method Blank ($pg\ L^{-1}$)	Detection Limit ($pg\ L^{-1}$)
2005	98 ± 13%	11 ± 8%	16.61	33.03
2006	103 ± 20%	6 ± 45%	85.04	139.65
2007	102 ± 19%	9 ± 9%	ND	30
2008	98 ± 10%	6 ± 6%	ND	30
2009	N/A	6 ± 8%	N/A	13

N/A: samples not analyzed
ND: non-detect

Table A3. QAQC results for Hg_T and MeHg analyses in solid phase samples, 2003-2009.

	MeHg	Hg_T
SRM Recovery (%)	92 ± 13%	97 ± 10%
Replicate RSD (%)	3 ± 4%	6 ± 6%
Spike Recovery (%)	N/A	94 ± 10

N/A: samples not analyzed

Table A4. %MeHg in porewater from each treatment within the S6 peatland (2006-2008). Measurements made within two weeks following a sulfate addition to the experimental treatment were not included in these calculations.

Treatment	Year	Minimum	Median	Maximum	90th Percentile
Control	2006-2008	0.3	4.0	41.1	10.7
Recovery	2006	2.8	14.5	31.0	22.7
Recovery	2007	0.1	10.0	33.5	20.1
Recovery	2008	1.3	8.5	41.6	19.9
Experimental	2006-2008	2.1	15.2	89.4	41.4

Table A5. Slope, intercept, and r^2 values for the linear relationships between porewater chemistry in lagg sites versus bog sites.

Analyte	Treatment	Slope	Intercept	r^2
Hg_T	Control	0.62	3.26	0.47
	Recovery	0.47	2.56	0.52
	Experimental	0.59	3.60	0.47
MeHg	Control	0.26	0.16	0.54
	Recovery	0.34	0.46	0.39
	Experimental	1.60	0.01	0.62
%MeHg	Control	0.30	2.02	0.23
	Recovery	0.93	3.11	0.61
	Experimental	1.14	5.06	0.41
SO_4^{2-}	Control	0.21	0.20	0.30
	Recovery	0.27	0.31	0.44
	Experimental	0.68	0.99	0.66
DOC	Control	1.36	-6.5	0.81
	Recovery	1.24	7.31	0.38
	Experimental	1.08	8.88	0.67

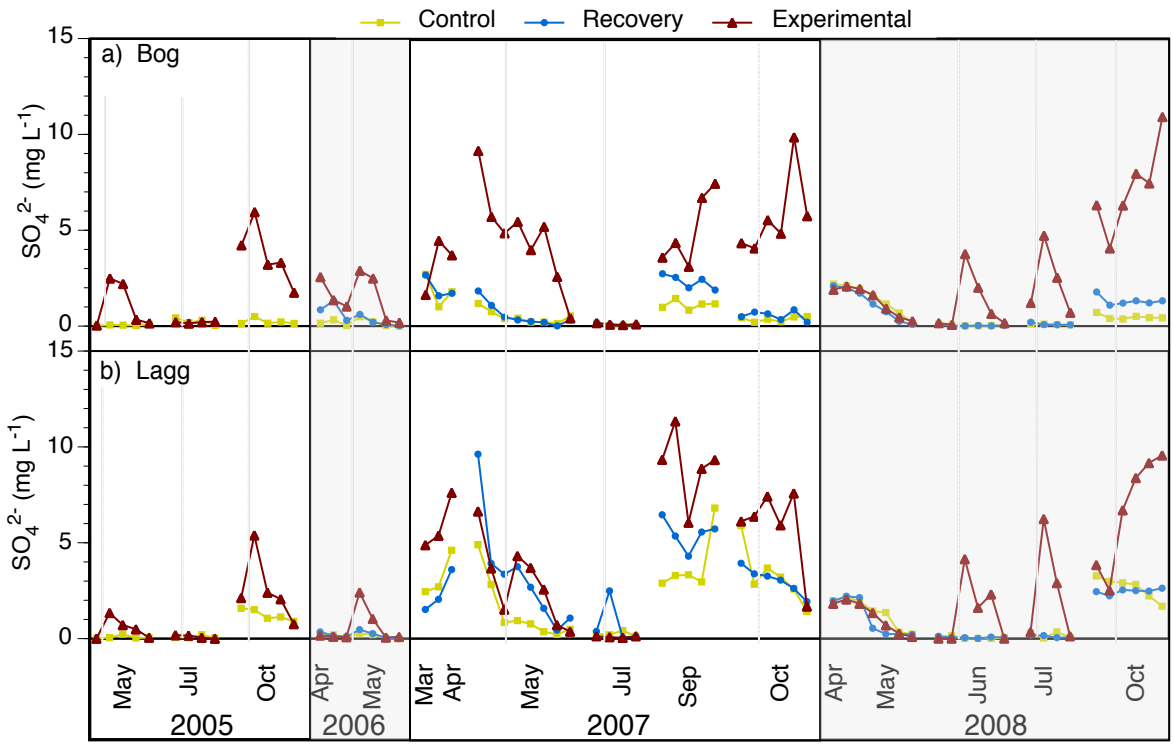

Figure A1. Sulfate concentrations in porewaters of bog and lagg sites in the control, recovery, and experimental treatments, 2005-2008. Dashed gray lines represent sulfate additions to the experimental treatment.

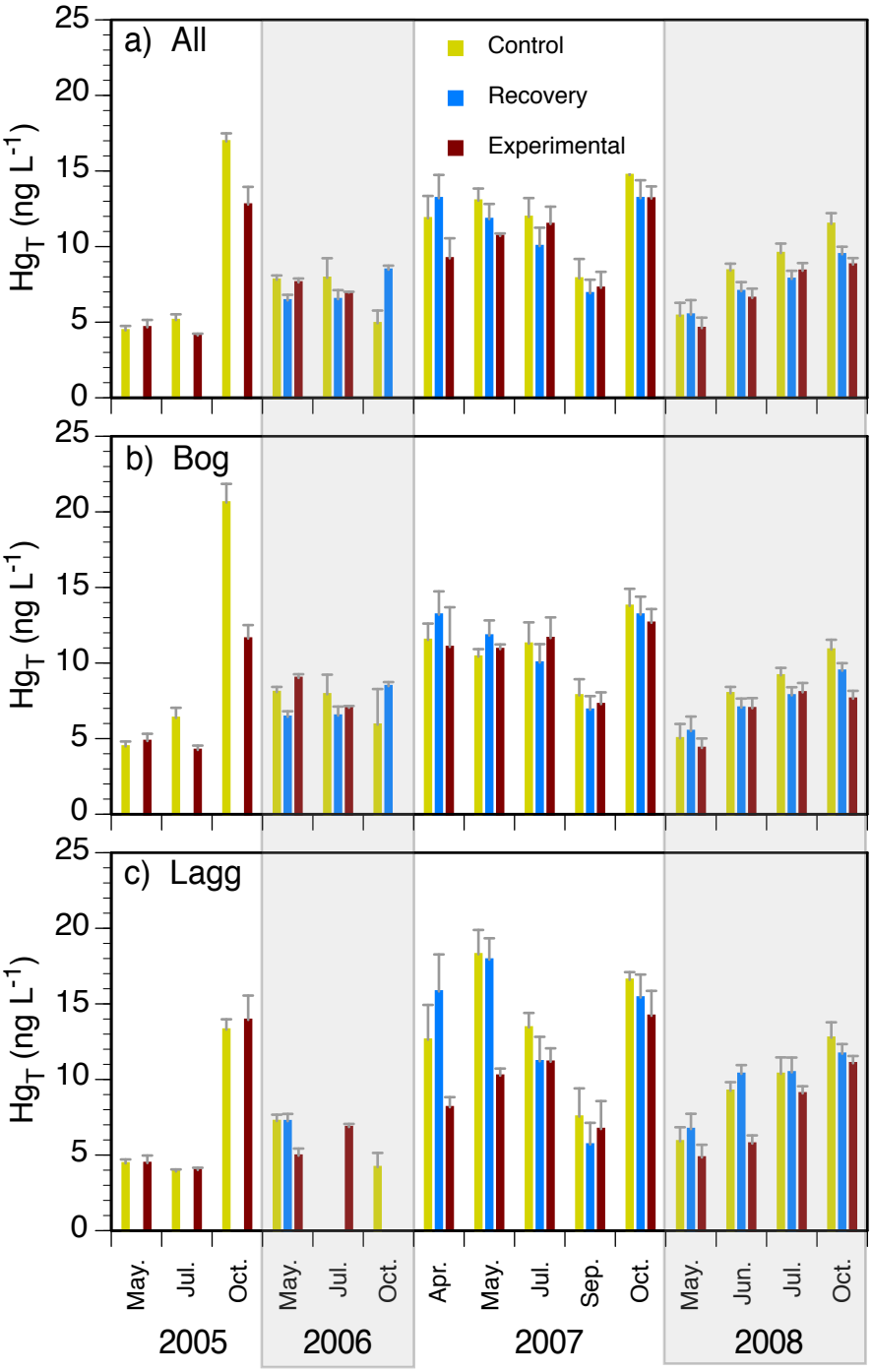

Figure A2. Average seasonal Hg_T concentrations in porewaters from all sites (a), the central bog (b), and the lagg margin (c) of the S6 peatland, 2005-2008.

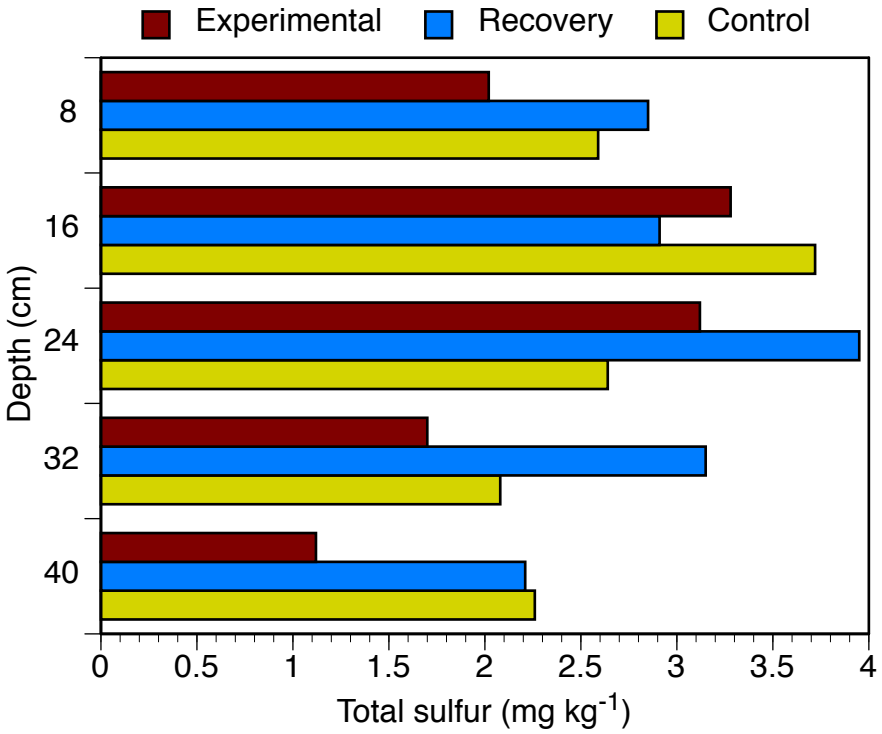

Figure A3. Solid-phase total sulfur concentrations at lagg sites from the control, recovery, and experimental treatments in October 2006.

Appendix B: Supporting information for Chapter 4

Table B1. Peat cores collected from the S6 peatland.

Year	Total Cores	Cores per Treatment	Intervals Sectioned (cm)	Coring Method
2003	12	6	0-4, 4-8	Polycarbonate tube and hand-collection
2005	10	5	0-2, 2-4, 4-8	Mechanical corer
2006	6	2	0-4, 4-8	Serrated knife and hand-collection
2007	6	2	0-4, 4-8	Serrated knife and hand-collection
2009	18	6	0-2, 2-4, 4-8	Polycarbonate tube and hand-collection

Table B2. Quality control and assurance results for 2005-2009 Hg_T analyses in porewaters.

	Percent Spike Recovery	Duplicate Relative Percent Difference	Method Blank (ng L^{-1})	Detection Limit (ng L^{-1})
2005	98± 6	2 ± 2%	0.22	0.31
2006	93± 14%	3 ± 7%	0.53	0.85
2007	78± 16%	8 ± 5%	0.90	1.10
2008	114± 17%	7 ± 7%	0.79	0.72
2009	92 ± 4	1 ± 1%	N/A	0.22

N/A: samples not analyzed

Table B3. Quality control and assurance results for 2005-2009 methylmercury analyses in porewaters.

	Percent Spike Recovery	Duplicate Relative Percent Difference	Method Blank (pg L^{-1})	Detection Limit (pg L^{-1})
2005	98 ± 13%	11 ± 8%	16.61	33.03
2006	103 ± 20%	6 ± 45%	85.04	139.65
2007	102 ± 19%	9 ± 9%	ND	30
2008	98 ± 10%	6 ± 6%	ND	30
2009	N/A	6 ± 8%	N/A	13

N/A: samples not analyzed
ND: non-detect

Table B4. Quality control and assurance results for 2003-2009 Hg_T and MeHg analyses in peat.

	MeHg	Hg_T
SRM Recovery (%)	92 ± 13%	97 ± 10%
Replicate RSD (%)	3 ± 4%	6 ± 6%
Spike Recovery (%)	N/A	94 ± 10

N/A: samples not analyzed

SRM was ERM-CC580 for MeHg; MESS-3 for Hg_T

Table B5. Parameters used for geochemical modeling in MINEQL. See table 5 in Skyllberg (2008) for log K values.

Parameter	Value
MeHg	3.1×10^{-9} M
Hg_T	2.7×10^{-8} M
RSH (dissolved)	3.2×10^{-6} M
RSH (solid)	9×10^{-4} M
pH	4.0

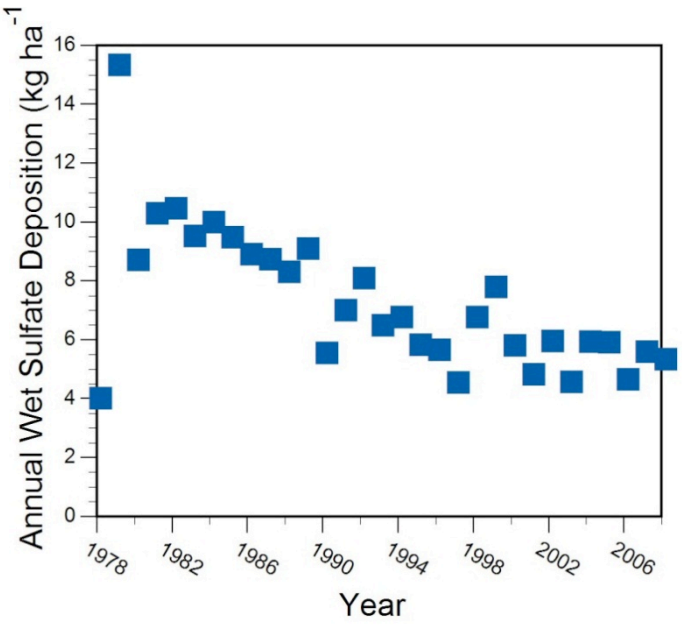

Figure B1. Annual wet sulfate deposition rates at the National Atmospheric Deposition Program site (MN-16) located at the Marcell Experimental Forest.

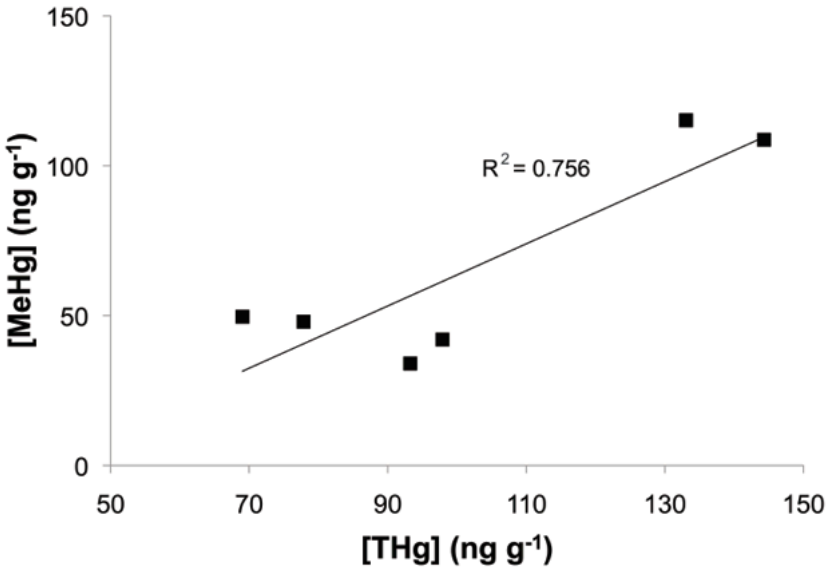

Figure B2. MeHg concentrations versus Hg_T concentrations in mosquito larvae collected from the S6 peatland in the spring of 2009. MeHg data were limited (n = 6) because of small sample mass.